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# *NEW VARIETIES OF WESTERN WILLOWS*

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Increasing collections of West American Salices make possible better interpretations of the variations of expression noted in different species. The present paper adds two varieties to *Salix melanopsis* Nuttall, that one of the western long-leaf or sandbar willows most closely related to the eastern *S. interior* Rowlee (*S. longifolia* Muhl.). Continuing study of the abundant Alaskan collections made by Dr. and Mrs. Setchell and others have revealed significant variations in *S. fuscescens* And., *S. reticulata* L. and *S. ovalifolia* Trautvetter. Descriptions of these as new varieties should make these species more readily comprehended.

Type specimens are indicated and available herbarium specimens are cited. The following initials are used to designate the herbariums in which the collections examined are housed: CAS, California Academy of Sciences; CRB, Carleton R. Ball; PANS, Philadelphia Academy of Natural Sciences; SU, Stanford University; UC, University of California; and USN, U. S. National Herbarium. The courtesies shown by the institutions named are appreciated, as is also the assistance received from Dr. Setchell and others.

SALIX MELANOPSIS variety GRACILIPES Ball, n. var.

Sectio Longifoliae. A forma typica per capsulas pedicellatas abludens.

This variety is distinguished by the distinctly pedicelled capsules. In the species the capsules are sessile in the upper part of the ament and borne on very short pedicels, usually about 0.3–0.5 mm. long, in the basal portion. The aments of the variety tend also to be laxer and in some cases longer also.

Aments of both sexes 3–6 or up to 9 cm. long, solitary or in pairs on leafy peduncles, more or less lax, the longer remotely flowered at the base; pistillate peduncles 2–5 cm. long in flowering and 4–8 cm. long when fruiting, and bearing 4–7 foliage leaves. Capsules 4–5 mm. long; pedicels 1–1.5 mm. long in the lower portion of the ament and 0.5–1 mm. long in the upper portion; stigma short, sessile, divided.

Like many varieties, this one sometimes grades more or less insensibly into the species. The means, and especially the extremes, however, are so striking that it seems desirable to designate them as representing an observable and measurable variation. With such action, students and collectors are less likely to be puzzled by inconsistencies in specific

descriptions and also are led to expect, and therefore to note, other similar variations in the plants they collect. The varietal name, *gracilipes*, refers to the slender and elongated pedicels of the capsules. The staminate and pistillate types, as noted under distribution, are both from the Smith River in Del Norte County, California.

*Distribution.*—This newly recognized variety is known only from Humboldt and Del Norte Counties, California, in the extreme northwestern part of the State. In Del Norte County, it has been found chiefly near the coast, in locations approximately at sea level, where it occupies river bars and banks composed of sand, gravel, and boulders, in association with *S. melanopsis* and *S. parksiana*. The Humboldt County locality is far inland. The following specimens have been examined. The initials used to designate the containing herbariums are explained at the beginning of the paper.

Del Norte Co. Common low shrub, 1–1.5 m. high, on gravel bars at margin of Smith River, at Roosevelt Highway Bridge, alt. about sea level, Harold E. Parks 0981 (CAS; CRB, 2 sheets; SU; UC, 2 sheets, and many other herbariums; aments long, lax); 0988 (CAS; CRB, 3 sheets; SU; UC, 4 sheets, and many other herbariums; aments short, lax), both May 21, 1931. Low shrubs, abundant in gravelly margin of Smith River, at Roosevelt Highway Bridge, elev. 15 m., Parks and Tracy 01008 (♀), 01012 (♀), 01021 (pistillate type collection, aments midlength, lax), 01022 (♀, aments long, lax), all May 30, 1931 (CAS, CRB, SU, UC, and many other herbariums); Tracy 10824 (probably from same plant as Parks 01021), September 24, 1932 (CRB, UC). Abundant low shrub, 2–3 m. high, on creek bank near Smith River, below Gasquets, elev. 90–120 m., Harold E. Parks and Joseph P. Tracy 0935 (staminate type collection), May 10, 1931 (CAS; CRB, type, 2 sheets; SU; UC, 2 sheets); Joseph P. Tracy 9357 from same plant (CRB, UC).

Humboldt Co. Along Willow Creek, near its mouth at Trinity River, alt. 600 feet, Joseph P. Tracy 3295 (♂ and ♀), July 4, 1911 (UC); Trinity River, near Willow Creek, alt. 500 feet, Tracy 10095, May 17, 1932 (UC). Tracy 12262, Mattole River near Petrolia, May 20, 1933, has long, slender, acute scales and may represent a hybrid.

SALIX MELANOPSIS variety TENERRIMA (Henderson) Ball, n. comb.

*S. longifolia tenerrima* Henderson in Bull. Torr. Bot. Club 27:354, 1900.

*S. tenerrima* (Henderson) Heller, Cat. No. Am. Plants, ed. 2, 4, 1900.

*S. fluviatilis tenerrima* (Henderson) Howell, Fl. NW. Am. 618, 1902.

*S. linearifolia* Rydberg, Fl. Colo. 94, 1906 (in part); Fl. Rocky Mts. 192, 1917 (in part).

*S. exigua tenerrima* (Henderson) Schneider, in Bot. Gax. 67:335, 1919.

From the synonomy given above it will be noted that this willow has enjoyed specific rank under two different names, and varietal rank as an appendage of three different species of the sandbar or longleaf willows, previous to its present assignment as a variety of *S. melanopsis* Nuttall. The reasons for the present arrangement are twofold. In the first place, the characters of its branchlets, leaves and capsules are those of *melanopsis*, with two exceptions, and not those of any of the other three species. Those two exceptions are narrower blades and longer capsule pedicels, which constitute its claim to varietal rank. In the second place, its geographic distribution is almost exactly that of *S. melanopsis*, but is wholly outside the ranges of *S. interior* (*S. longifolia*) and *S. fluviatilis*, and coincident with that of *S. exigua* in only a small part of the area occupied by that widely-distributed species. The equivalent form of *S. exigua* is variety *stenophylla* (Rydberg) Schneider and the corresponding narrow-leaved expression of *S. interior* Rowlee is the variety *pedicellata* (Andersson) Ball.

Variety *tenerrima* differs from the species in linear leaves, 4–7 or 8 cm. long by 2–4 or 5 mm. wide on fruiting branchlets, 8–9 cm. by 5–7 mm. up to 11 cm. by 7 mm. on seasonal shoots, and 12–14 cm. by 10–13 mm. on sprouts, all more or less denticulate or

spinulose-denticulate; stipules obsolete or 1–4 mm. long on seasonal shoots, and up to 5–8 mm. long on sprouts, lanceolate, usually denticulate; pedicels of the capsules somewhat longer than in the species, 0.5–1 mm. long. From the other distinctly pedicelled variety, *S. melanopsis* var. *gracilipes* Ball, variety *tenerrima* differs in its very narrow blades.

*Distribution.*—Distributed sparingly with the species from southern British Columbia and Alberta south in Idaho to western Montana and western Wyoming, northern Utah, eastern portions of Washington and Oregon, and the Sierra Nevada in California. The following specimens have been examined recently. The initials designating containing herbariums are explained at the beginning of the paper.

*Wyoming:* Evanston, elev. 6750 ft., Geo. L. Fisher 65, June 17, 1929. Banks of Gros Ventre River, Jackson's Hole (Teton Co.), 10 ft. high, Merrill and Wilcox 996, July 14, 1901 (CRB, USN).

*Idaho:* Indian Reservation, middle of Bannock Valley, opposite Bannock Pk., 10 ft. tall, C. R. Ball 2021, August 6, 1915 (CRB, USN). Cache National Forest, Fish Haven Creek, elev. 6500 ft., Grazing Reconnaisance 331, August 15, 1916 (CRB). Bush 12–14 ft., near Rattlesnake Creek, alt. 3500 ft., L. F. Henderson 3153, July 11, 1895 (CRB, USN).

*California:* Butte Co., on banks of Butte Creek, Chico, E. B. Copeland, May 29, 1903, determined by von Seemen as "S. longifolia Muhl., form" and distributed by C. F. Baker as No. 3278 of Plants of the Pacific Coast (UC). Placer Co., French Meadows, Middle Fork of American River, Sec. 16, Twp. 15N., R. 14E., L. S. Smith 2627b, August 27, 1931 (CRB). Tulare Co., west edge of Sequoia National Park, among boulders in Kaweah River below Ash Mtn., alt. 450 m., Parks and Parks 01040, June 20, 1931 (CRB, UC); between Ash Mtn. and Three Rivers, alt. 300 m., Parks and Parks 01068a, September 28, 1931 (CRB, UC).

## SALIX FUSCESCENS variety REDUCTA, n. var.

Sectio Roseae. A forma typica per folia obovato-ovalia, basi rotundata aut solummodo subcuneata, per amenta breviora, et capsulas breviores (4–6 mm. longas), abludens.

Plant depauperate, prostrate; branches shorter, mostly less than 10 cm. long; leaves more crowded, petioles 1–2 mm. long, blades smaller, obovate-oval to oval or rarely suborbicular, scarcely cuneate, entire to minutely denticulate near base, 8–12 or 15, or rarely 20 mm. long, 7–11 mm. wide, common sizes being 11 × 7.5, 13 × 9 and 15 × 11 mm.; peduncles shorter, 1–2 cm. long; aments denser, projecting but slightly above the foliage shoots (as in *S. ovalifolia*) 1–2 or 2.5 cm. long, 1 cm. wide or rarely 4 by 1.3 cm.; scales oblanceolate-obovate; capsules lanceolate or broadly lanceolate, obtuse, commonly 4, sometimes 5, or rarely 6 mm. long, pedicels 1–1.5 mm. long; styles short, entire or sometimes divided.

Variety *reducta* is distinguished from the species by the smaller, obovate-oval blades, rounded or only subcuneate at base, and the shorter aments and capsules, the latter 4–6 mm. long, whereas those of *fuscescens* are 6–8 or 9 mm. long. It bears a deceptive resemblance to small-leaved specimens of *S. ovalifolia* or of its variety *cyclophylla*.

*Distribution.*—Variety *reducta* has been found once in the Alaska Range of southeastern Alaska, and more commonly in west-central Alaska in the vicinity of Norton Sound and Seward Peninsula, on St. Lawrence and St. Matthew Islands in Bering Sea, and at Plover Bay in eastern Siberia.

The species, *fuscescens*, ranges in America from Kodiak Island and the Alaska Peninsula to the Yukon Delta, Norton Sound and Seward Peninsula. It also occurs, but apparently less abundantly, across north-central Alaska, having been collected at Fairbanks, at Sourdough Creek on the Steese Highway from Fairbanks to Circle City,

and north-westward on the headwaters of the Kobuk River. Apparently the species does not occur in Bering Sea.

The following specimens have been examined. The initials used to designate the containing herbariums are explained at the beginning of the paper.

*Southeastern Alaska:* Alaska Range, near Paxson (Upper Copper River), F. W. Went S6, July, 1934 (CRB, UC).

*West-Central Alaska:* Sandy silt soil in wet tundra, Kotlik, Charles H. Rouse 33, July 7, 1931 (CRB). Seward Peninsula, Nome, F. E. Blaisdell, in 1900 (UC). Norton Sound: St. Michaels, J. C. Russell, July 10, 1889 (USN, this fragment differs by larger blades, up to 2 cm. long, narrowly oval and denticulate, and capsules to 6 mm. long).

*Bering Sea:* St. Lawrence Island, Northeast Cape, Coville and Kearney 2002, July 13, 1899 (USN). St. Matthew Island, Coville and Kearney 2087a, July 15, 1899 (CRB, USN, pistillate type). Punuk Islands (at south-east end of St. Lawrence Island, Latitude 64°), Otto Wm. Geist S 5A, August 15–September 3, 1934 (CRB, UC). Geist S 3 and S 4 from same locality may belong here but leaves are mostly gone.

*Alaska, Locality Unknown:* Roland Snodgrass 36, 65, in 1933 (Alaska Agricultural College).

*Northeastern Siberia:* Plover Bay (Lat. 64° N.), L. J. Cole (Harriman Expedition), July 11, 1899 (USN); Coville and Kearney 1856, July 11, 1899 (USN).

## SALIX OVALIFOLIA var. CYCLOPHYLLA (Rydberg) n. comb.

*Salix cyclophylla* Rydberg in Bul. N. Y. Bot. Gard. 1:274–275, 1899. (Rydberg here makes no mention of the untenable name, *S. rotundata*, given by him to Macoun for the same specimens and published by the latter (see below) in the same year.)

*S. ovalifolia* (in part) of Trautvetter and recent authors, as Coville, The willows of Alaska, Proc. Washn. Acad. Sci. 3:331–333, fig. 26, 1901, where mature leaf *d* represents this variety.

*S. rotundata* Rydberg (mss. name used without description) in James M. Macoun, A list of the plants of the Pribilof Islands, Bering Sea, with notes on their distribution (pp. 555–587), in David Starr Jordan, The fur seals and fur-seal islands of the North Pacific Ocean 3:571, 1899, not Forbes 1829.

Prostrate shrub; branchlets slender, yellowish, glabrous; leaves exstipulate, petioles up to 7 mm. long, blades suborbicular, oblate-orbicular, or broadly obovate-oval, the terminal sometimes cordate-ovate, 12–20 or rarely 25 mm. long, and of the same width, sizes observed (length-width) being 12 by 13, 14 by 15, 16 by 17, 17 by 15–17, 18 by 16, 19 by 17, 20 by 20, and 25 by 25 mm., the base broadly rounded to retuse, the apex similar; scales dark brown to black, obovate, thinly pilose; capsules stoutish, lanceolate to subovate, 5–6 mm. long, often obtuse at apex; brown, often glaucous, glabrous or rarely thinly pubescent; styles 0.5 to rarely 0.8 mm. long, entire or somewhat divided; stigmas about 0.5 mm. long, divided.

Variety *cyclophylla* differs from the species chiefly in the suborbicular instead of oval leaves. In general the plant is smaller in all its parts. The leaves of the species often reach 3.5–4 cm. in length and the capsules 7–8 mm. in length.

*Distribution.*—Variety *cyclophylla* has been found only on the Shumagin Islands, near the juncture of the Alaska Peninsula and the Aleutian Chain, and on the island groups of Bering Sea and Bering Strait. Some specimens from Arctic Alaska in the vicinity of Point Hope and Point Barrow approach this variety in leaf shape. The specimens listed below have been examined and the initials of containing herbariums are explained at the beginning of the paper.

*Shumagin Islands,* Popof Island, Trelease and Saunders 3425, July 7 to 18, 1899 (USN).

*Bering Sea, Pribilof Islands* (*St. George and St. Paul*). Seal Islands, Mrs. Capt. Bryant, in 1875 (USN). St. George Island, Garden Cove ridge, E. C. Johnston, July 6, 1924 (CRB). St. Paul Island: Brewer and Coe 325, 329, July 9, 1899 (USN); L. J. Cole, July 9, 1899 (USN); George Haley, summer of 1925 (UC); east outlet of Polovina crater, E. C. Johnston, June 14, 1925 (CRB); Trevor Kincaid, July 29, 1897; Jas. M. Macoun, August 9, 1914 (CRB, NMC 93813, UC); August 16, 1914 (CRB, NMC 93814, UC), June 29, 1914 (CRB, NMC 93815, 93816, UC), August 1, 1897 (USN, sheet 338581, probably a cotype of *S. cyclophylla*); Wm. Palmer 353, 424, June 21 and July 2, 1890 (USN); Wm. Palmer 556 (FVC), July, 1890 (USN); C. H. Townsend (Coll. of the U. S. Fish Comm. Str. Albatross), August, 1893 (USN); A. G. Whitney, 1912–1914 (USN).

*Bering Sea, Hall and St. Matthew Islands.* Hall Island, Coville and Kearney 2084, 2085, July 14, 1899 (USN, 2 sheets of each). St. Matthew Island, Coville and Kearney 2087 (in part), July 15, 1899 (CRB, USN, both in part, mixed with the species); Brewer and Coe 469, prostrate, July 15, 1899 (USN); G. D. Hanna, July 8–13, 1916 (USN, 4 sheets).

*Bering Strait, St. Lawrence Island*, Northeast Cape, Coville and Kearney 2002a, July 13, 1899 (USN, taken out of 2002); Iviktook Lagoon, H. L. Mason 6101, 6102, 6106, July 10, 1931 (CRB, UC, the first two distributed as *S. ovalifolia subarctica* Lundstr.); Near cliff east of Savoonga, Otto Wm. Geist, July 17, 1931 (CRB, UC, capsules very short and blunt, ovate-oval, styles extremely short, distributed as *S. ovalifolia* or *X. fuscescens*)

## SALIX RETICULATA var. GIGANTIFOLIA, n. var.

Sectio Reticulatae. A forma typica per folia majora (vulgo 4–6 cm. longa), per amenta longiora, et per capsulas longiores abludens.

Similar to *S. reticulata* but larger in every way; branches to 10 cm. long; leaves large, long-petioled; petioles mostly 2–4 or 4.5 cm. long; blades obovate-oval to suborbicular or oblate-orbicular, mostly 4–6 cm. long, 3.5–5 or 5.6 cm. wide, common sizes being 4 by 3.3, 4.5 by 4.5, 5 by 3.3–3.5, and 6 by 4.5 cm., rarely wider than long and then 5 by 4.8 and 5.6 by 5.2 cm., respectively, obtuse to retuse at apex, rounded to retuse or cordate at base, the larger with margins glandular-crenate throughout. Aments leafy-pedunculate, 2.5–4 or 5 cm. long, 1 cm. wide, lax in the basal portion; flower scales broadly oblanceolate or obovate, or almost triangular, broadly rounded or truncate at apex, 1.5–2 or 2.5 mm. long, thinly pilose to glabrate outside; capsules 4–5 mm. long, oblong or ovate-oblong, obtuse, subsessile or the pedicel up to 0.4 mm. long; styles and stigmas 0.2–0.4 mm. long.

This variety differs chiefly from the species and from var. *subrotunda* in the larger leaf-blades, mostly 4–6 cm. long, and the larger aments and longer capsules. In the field and herbarium it is a most striking plant. The pistillate type is F. A. Walpole 1181 from Kodiak Mountain, Kodiak Island, in the U. S. National Herbarium, Sheets Nos. 378139 and 378140. The staminate type is W. A. and C. B. Setchell 107a, Isabella Pass, Alaska Range, in the Herbarium of the University of California, Sheet No. 451086.

*Distribution.*—Variety *gigantifolia* is distributed in southern and south-central Alaska, along or near the Alaska range from Mt. McKinley National Park and the Copper River area southwest along the Alaska Peninsula to Unalaska. It seems to be most abundant in the central portion of this range, in the area covered by Kadiak Island and the upper Alaska Peninsula. The following specimens have been examined. The containing herbariums and the initials used to designate them are given at the beginning of this paper.

Alaska Range, Isabella Pass, elev. 3310 ft., W. A. and C. B. Setchell 107a (staminate type), June 27, 1931 (UC). Mt. McKinley National Park, east end not far from Super-

intendent's headquarters, elev. 3000 to 3300 ft., David Kaye 2, 3, in 1932 (CRB, UC). Copper River Region, trailing, 2.5 inches high, common in high tundra, West Fork of Eagle Creek, alt. 4500 ft., Wm. L. Pope 160, Aug. 22, 1902 (USN).

Kadiak Id.: Kadiak, L. J. Cole, July 1, 1899 (USN); Coville and Kearney 2312, July 20, 1899 (USN); C. V. Piper 4592, July 28, 1904 (CRB, USN). Kadiak Mountain, F. A. Walpole 1181 (pistillate type), July 30, 1900 (CRB, USN). Alaska Peninsula: Kukak Bay, Coville and Kearney 1537, July 1–5, 1899 (USN); Katmai Region, A. E. Miller, July 21, 1919 (USN).

Shumagin Ids.: Unga, F. A. Golder 40, Sept. 20, 1899 (USN). Unalaska: (U. S. Coast Survey Expedition to Alaska, 1867, under George Davidson), Dr. A. Kellogg 325 (PANS); Amakaak Id., at Unalaska, B. W. Evermann 156, Aug. 2, 1892 (USN).

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## *THE OXYGEN EQUILIBRIUM OF HEMOGLOBIN AND ITS STRUCTURAL INTERPRETATION*

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The work of the early investigators of blood (Bohr, Hasselbalch, Krogh, Barcroft, Haldane, Henderson) showed that the oxygen equilibrium of hemoglobin is affected by the hydrogen ion concentration of the solution, and, moreover, that it does not correspond to the simple equilibrium $Hb + O_2 \rightleftarrows HbO_2$ (in which the symbol Hb represents the portion of the hemoglobin molecule containing one heme and capable of combining with one oxygen molecule). After completing his osmotic pressure experiments showing that the hemoglobin molecule contains four hemes, Adair[1] suggested that the $Hb_4$ molecule adds four oxygen molecules successively, with different equilibrium constants. He and Ferry and Green[2] found that their data on the oxygen equilibrium of hemoglobin solutions at constant pH can be represented by a four-constant equation corresponding to this conception, the values of the constants changing in a regular way with changing pH.

On the basis of certain postulates regarding the structure of the hemoglobin molecule, I have derived an equation involving only two constants which satisfactorily represents the data on the oxygen equilibrium at constant pH, and also an equation involving two constants which represents the change in the oxygen equilibrium with change in pH. The validity of these equations provides considerable support for a particular structure of the molecule.

The most accurate and extensive data on the oxygen equilibrium are those obtained by Ferry and Green[2] for horse hemoglobin in phosphate and borate buffers. Ferry and Green remarked that their curves of $y$ (the ratio

$O_2$ absorbed/$O_2$ content at saturation) versus $p$ (the oxygen pressure) at different values of pH can be superimposed by making a change of scale for $p$. On doing this, the experimental points all fall near a smooth curve, as shown in figure 1. This curve can be reproduced roughly by the equation

$$y = \frac{Kp^n}{1 + Kp^n} \tag{1}$$

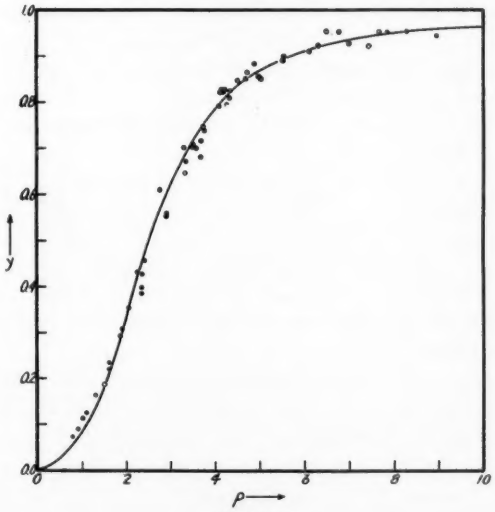


FIGURE 1

The experimental values of the fractional absorption of oxygen, $y$, plotted against the oxygen pressure $p$ (the points obtained at various pH values being reduced to pH 8.30 by a change of scale in $p$), compared with the theoretical curve of Equation 3, corresponding to the square structure of the hemoglobin molecule.

which corresponds to the equilibrium $Hb_n + n\ O_2 \rightleftarrows Hb_n(O_2)_n$, the value of $n$ being about 2.6. The curve can be accurately represented by Adair's four-constant equation

$$y = \frac{K_1p + 2K_2p^2 + 3K_3p^3 + 4K_4p^4}{4(1 + K_1p + K_2p^2 + K_3p^3 + K_4p^4)}, \tag{2}$$

and also by the two-constant Equation 3, which we shall now derive.

Let us make the following postulates regarding the structure of the hemoglobin molecule.

1. The hemoglobin molecule contains four hemes, each of which is

connected with $k$ others, the connection being such as to permit the interaction of the hemes in pairs.

2. The four hemes are equivalent.
3. The interactions for connected pairs are equivalent.

We distinguish four cases, corresponding to $k = 0$, 1, 2 and 3.

No interaction of hemes, $k = 0$. This leads to Equation 1 with $n = 1$, and is hence not compatible with experiment.

Interaction of the hemes in pairs $\begin{pmatrix} \text{Hb} & \text{Hb} \\ | & | \\ \text{Hb} & \text{Hb} \end{pmatrix}$, $k = 1$. This leads to an equation which can be approximated by Equation 1 with $1 \leqslant n \leqslant 2$, and is hence not compatible with experiment.

Hemes at the corners of a square, interactions along the sides of the square $\begin{pmatrix} \text{Hb—Hb} \\ | \quad | \\ \text{Hb—Hb} \end{pmatrix}$, $k = 2$. This leads to Equation 3, in agreement with experiment.

Hemes at the corners of a tetrahedron, interactions along the edges of the tetrahedron, $k = 3$. This leads to Equation 4, in agreement with experiment; however, for other reasons the structure is less satisfactory than the square structure.

To derive Equation 3 on the basis of the square arrangement, we note that at the oxygen pressure $p$ the relative amounts of the six molecular species present are the following:

| Hb—Hb<br>\| \|<br>Hb—Hb | Hb—$HbO_2$<br>\| \|<br>Hb—Hb | Hb—$HbO_2$<br>\| \|<br>Hb—$HbO_2$ | Hb—$HbO_2$<br>\| \|<br>$HbO_2$—Hb | Hb—$HbO_2$<br>\| \|<br>$HbO_2$—$HbO_2$ | $HbO_2$—$HbO_2$<br>\| \|<br>$HbO_2$—$HbO_2$ |
|---|---|---|---|---|---|
| 1 | $4K'p$ | $4\alpha K'^2p^2$ | $2K'^2p^2$ | $4\alpha^2K'^3p^3$ | $\alpha^4K'^4p^4$ |

The constants $K'$ and $\alpha$ have the following significance, $RT \ln K'$ is the free energy change accompanying the addition of oxygen to heme, and $RT \ln \alpha$ is the additional free energy stabilizing two interacting $HbO_2$ groups; that is, the free energy is decreased by $RT \ln \alpha$ for each interaction shown by a heavy bar. The factors 2 and 4 result from the symmetry numbers.

These relative amounts $1:4K'p:(4\alpha + 2)\ K'^2p^2:4\alpha^2K'^3p^3:\alpha^4K'^4p^4$ of the substances $Hb_4$, $Hb_4O_2$, $Hb_4(O_2)_2$, $Hb_4(O_2)_3$ and $Hb_4(O_2)_4$ lead directly to the following equation for $y$:

$$y = \frac{K'p + (2\alpha + 1)K'^2p^2 + 3\alpha^2K'^3p^3 + \alpha^4K'^4p^4}{1 + 4K'p + (4\alpha + 2)K'^2p^2 + 4\alpha^2K'^3p^3 + \alpha^4K'^4p^4}. \quad (3)$$

The curve shown in figure 1 is calculated by means of this equation, with $\alpha = 12$ and $K' = 0.033$. It is seen that the agreement with the experimental points is satisfactory. (The uniform deviation shown by the first

five points may be due to experimental error, or may be real, indicating that the theory is only approximate.)

The similar discussion of the tetrahedral configuration leads to the equation

$$y = \frac{K'p + 3\alpha K'^2p^2 + 3\alpha^3K'^3p^3 + \alpha^6K'^4p^4}{1 + 4K'p + 6\alpha K'^2p^2 + 4\alpha^3K'^3p^3 + \alpha^6K'^4p^4}, \qquad (4)$$

in which $K'$ and $\alpha$ have the same significance as before. This equation

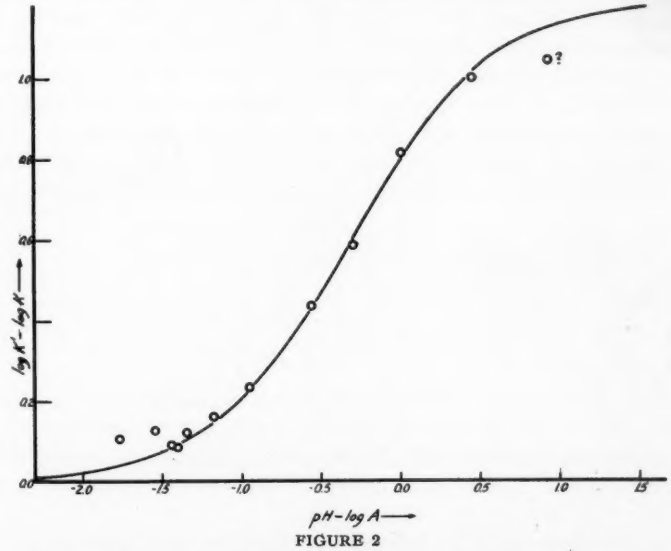


FIGURE 2

The dependence of the equilibrium constant $K'$ of Equation 3 on pH. The circles are experimental values, and the curve is drawn according to Equation 6.

provides a curve almost indistinguishable from the one shown in the figure, the value of $\alpha$ being about $12^{2/3} = 5.3$.

Although the tetrahedral configuration is thus compatible with the oxygen equilibrium data, we reject it in favor of the square configuration for the following reasons. The hemoglobin molecule is roughly spherical in shape, with a radius of about 29 Å, and it is very probable that the hemes are located on the surface of the molecule; if arranged tetrahedrally, they would be about 47 Å apart. It is very difficult to imagine a connection between two hemes this far apart which would lead to an interaction energy as large as $RT \ln \alpha = 1000$ cal./mole. Moreover, the tetrahedral configuration would lead one to expect a three-fold axis of symmetry for

the hemes rather than the four-fold axis possessed by the porphyrin nucleus.

To account for the dependence of the oxygen equilibrium on the hydrogen ion concentration, we give quantitative expression to the familiar qualitative explanation that there is an interaction between the oxygen molecules of oxyhemoglobin and some acid groups. The fact that an equation of the form of 3 can be made to account for the data for various pH values by changing $K'$ alone indicates strongly that each heme contains its own acid groups, the interaction of one heme with the acid groups of another being negligible.[3] Let us first assume that there is one acid group for each heme, with interaction constant $\beta$ such that the free energy change on dissociation of $HHbO_2$ to $H^+$ and $HbO_2^-$ is $RT \ln \beta$ less than that of HHb to $H^+$ and $Hb^-$, oxyhemoglobin being a stronger acid than hemoglobin. The relative amounts of the four molecular species (considering only one heme) HHb, $Hb^-$, $HHbO_2$ and $HbO_2^-$ are then $1 : A/[H^+] : Kp : Kp\beta A/[H^+]$, and the oxygen equilibrium formula becomes

$$y = \frac{K'p}{1 + K'p}$$

with

$$K' = K \frac{(1 + \beta A/[H^+])}{(1 + A/[H^+])}, \qquad (5)$$

in which $A$ is an acid-strength constant. It is found on similarly considering the 55 molecular species $H_4Hb_4$, $H_4Hb_4O_2$, etc., corresponding to the square arrangement that the oxygen equilibrium is expressed by Equation 3, with $K'$ dependent on the hydrogen ion concentration in the way given by Equation 5.

The dependence of $K'$ on pH as found experimentally from the factors used in reducing the points of figure 1 to a single curve is shown[4] in figure 2. It is found that Equation 5 does not represent this dependence satisfactorily. If, however, we assume that there are two equivalent acid groups interacting with each heme, we derive the equation

$$K' = K \frac{(1 + \beta A/[H^+])^2}{(1 + A/[H^+])^2}, \qquad (6)$$

which does agree satisfactorily with experiment,[5] as shown by the curve in the figure, calculated for $\beta = 4$, $-\log A = 7.94$ and $K = 0.0035$.

We conclude that the data on the oxygen equilibrium of hemoglobin indicate that the four hemes of the molecule are arranged at the corners of a square; each heme is connected with two others in such a way as to give rise to an interaction energy of $RT \ln \alpha = 1500$ cal./mole for each pair of adjacent oxyhemes, and each heme contains two acid groups, the acid

group-oxyheme interaction energy being $RT \ln \beta = 820$ cal./mole. The total oxyheme-oxyheme interaction energy is about 6000 cal./mole, and the total acid group-oxyheme interaction energy is about 6600 cal./mole.

The none too exact experiments on the carbon monoxide equilibrium and the carbon monoxide-oxygen balance of hemoglobin suggest that the interactions $\alpha$ and $\beta$ are effective for carboxyhemoglobin as well as for oxyhemoglobin. On the other hand, the results of Anson and Mirsky[6] on the carbon monoxide equilibrium of hemochromogen, which agree with Equation 1 with $n = 1$, show that there is no heme-heme interaction in the hemochromogen studied. It seems not improbable that the hemochromogens differ from hemoglobin mainly in that in the hemochromogens the hemes are independent and in hemoglobin four hemes form a conjugated system.

[1] G. S. Adair, *Proc. Roy. Soc.*, **A109**, 292 (1925); *Jour. Biol. Chem.*, **63**, 529 (1925).

[2] R. M. Ferry and A. A. Green, *Jour. Biol. Chem.*, **81**, 175 (1929).

[3] It is possible that the results of more accurate experiments would require this conclusion to be revised.

[4] The points for pH 5.8, 4.9 and 4.5 are not shown. These are obtained from only one or three measurements; they lie somewhat above the origin (0.1–0.2), which may indicate that some other interaction becomes effective in very acid solutions.

[5] We have neglected the mutual interaction of the two acid groups, the data not being sufficiently accurate and extensive to make a discussion of this interaction profitable.

[6] M. L. Anson and A. E. Mirsky, *Jour. Physiol.*, **60**, 50 (1925).

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## *THE RATE AND EXTENT OF ANAEROBIC DECOMPOSITION OF SULFITE WASTE LIQUOR BY BACTERIA OF SEA BOTTOM MUD*

### *II. BACTERIOLOGICAL*

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In Part One of this paper, to which the reader is referred,[1] the experimental set-up and procedure, chemical methods and results of chemical analyses were described in detail. Reviewing briefly, gallon bottles were filled about one-third full of marine mud and the rest of the bottle was filled with sea water, to which various amounts of the sulfite waste liquor (s.w.l.) had been added. The bottles were then incubated at 9°, 21° and 36°C.; the evolved gases were collected, measured and analyzed chemically. During the 310 day incubation period through which fermentation

was allowed to proceed, bacterial counts were periodically made, and toward the end of the work a number of organisms were isolated in pure culture and their characteristics studied in detail.

*A. Bacterial Counts.—Media.*—Since marine mud and sea water were used in the study, a medium suggested by Lippman[4] for marine bacteria was at first used:

| | |
|---|---|
| Calcium lactate | 1 gr. |
| Peptone | 1 gr. |
| $KNO_3$ | 0.5 gr. |
| Agar | 15 gr. |
| Sea water | 1000 cc. |

pH—8.0

The first plating (after three days) before the appearance of any gas gave a high bacterial count. However, as the rate of fermentation, as measured by the rate of evolution of gas, began to increase the counts made with the above medium, instead of showing the expected increase, rapidly decreased, indicating that for the type of flora that was developing the above medium was unsuitable. After some experimentation, during which some twelve different compositions were tried, the following was adopted and used for the rest of the counts:

| | |
|---|---|
| Peptone | 5.0 gr. |
| Dextrose | 10.0 gr. |
| $KNO_3$ | 0.5 gr. |
| $K_2HPO_4$ | 0.5 gr. |
| Agar | 18 gr. |
| Sea water | 1000 cc. |

pH—6.8

The addition of 10 to 20 per cent. of s.w.l. to the agar media did not prove to be of value and counts obtained with such media were as a rule smaller than when the above medium, containing no s.w.l., was used.

*Plates.*—Plates were incubated under both aerobic and anaerobic conditions. The anaerobiosis was obtained by means of Spray[6] dishes. Consistent anaerobic counts were not obtained by this method for, although the alkaline pyrogallol is a very efficient oxygen remover, the paraffin seal of pure paraffin or of paraffin softened by the addition of petrolatum, could not always withstand the air pressure resulting from the partial vacuum in the dish due to oxygen absorption, and leaked. Occasionally the dish itself became cracked by the external air pressure. By placing vials containing methylene-blue indicator in the Spray dishes it was shown that on the average in about half of the dishes anaerobiosis was not complete. The presence of strong alkali in the dish also caused a complete removal of carbon dioxide from the air, which gas, if not essential, is at least conducive to the growth of many bacteria.

On account of the unreliability of the method used the anaerobic counts

TABLE 1

AEROBIC BACTERIAL COUNTS IN THOUSANDS PER CUBIC CENTIMETER

| INCUBATION T° | BOTTLE NO. AND % S.W.L. PRESENT | LIPPMAN'S AGAR | | GLUCOSE AGAR FERMENTATION TIME IN DAYS | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 3 | 43 | 72 | 86 | 103 | 115 | 135 | 151 | 169 | 189 | 204 | 224 | 242 | 263 | 314 |
| 9°C. | A—Blank | 171 | 304 | 510 | 790 | 810 | 200 | 123 | 700 | ... | 500 | 760 | ... | 436 | 520 | 1730 |
| | B—1.4% | 2680 | 100 | 220 | 272 | 82 | 136 | 490 | 451 | 215 | 480 | 137 | ... | 140 | 490 | 960 |
| | C—5.3% | 9000 | 279 | 540 | 243 | 440 | 207 | 236 | 474 | 156 | 860 | 101 | ... | 750 | 570 | 900 |
| | D—16.4% | 12600 | 1130 | 1110 | 750 | 790 | 760 | 102 | 760 | 950 | 880 | 800 | 850 | 450 | 1210 | 1780 |
| 21°C. | A—Blank | 112 | 770 | 780 | ... | 68 | 76 | ... | 58 | ... | 217 | 209 | ... | 166 | 59 | 77 |
| | B—1.4% | 211 | 810 | 100 | 177 | 91 | 420 | 225 | 143 | 122 | 161 | 222 | ... | 131 | 370 | 380 |
| | C—5.3% | 7200 | 650 | 570 | 310 | 550 | 189 | 218 | 480 | ... | ... | 400″″ | ... | ... | ... | 1030″″ |
| | D—16.4% | 9900 | 138 | 2650 | 2070 | 1570 | 660 | 510 | 1050 | 490 | 1200 | 300 | 620 | 730 | 560 | 930 |
| | E—26.4% | 1190 | 101 | 650 | 350 | 350 | 460 | 500 | 3800 | 830 | 420 | 230 | 260 | 321 | 150 | 370 |
| 36°C. | A—Blank | 31 | 30 | 105 | ... | 115 | 49 | 0* | 100** | 1020″ | 1160 | 146 | 50 | 60 | 60 | 65 |
| | C—5.3% | 9500 | 20 | 108 | ... | 101 | 135 | 2* | 62** | 229″ | 77 | 146 | 71 | 42 | 79 | 208 |
| | D—16.4% | 5000 | 23 | 340 | ... | 18 | 38 | .4* | 22** | 360″ | 62 | 302 | 90 | 23 | 12 | 21 |
| | E—26.4% | 2000 | 21 | 71 | ... | 177 | 440 | 0* | 32** | 860″ | 169 | 410 | 50 | 200 | 197 | 153 |

* The 36° thermostat became overheated on the 125th day, killing most of the bacteria.

** Inoculated five days before with ten cc. from "D" at 21°.

″ Re-inoculated from "D" at 21°C.

″″ Count made from duplicate bottle used for gas studies, original bottle broken on 152nd day.

are omitted from this paper. We have since discontinued the use of Spray dishes and in all anaerobic work used McIntosh-Fildes jars exclusively.

*Incubation and Counting.*—All counts were made from plates incubated at room temperature. Duplicates placed in incubators at 9°C. and 36°C. gave identical counts. The colonies were counted after three weeks with the aid of a dissecting microscope.

*Discussion of Count Data.*—Lack of space prevents giving detailed correlation of bacterial counts with chemical data presented in Part One[1] to which the reader is again referred.

We may note from the table that at first the addition of s.w.l. acted as a stimulant to bacterial growth: compare "3 days" blanks "*A*" with the others. At the end of 43 days the number of viable bacteria in each bottle had decreased and remained practically constant for the rest of the experiment. This suggests that a certain state of equilibrium was attained between the various physiologically different bacteria present. The change of medium between the "43 days" and "72 days" counts had no effect on the counts in the bottles in which only a small amount of fermentation took place (all 9° and the first three of the 21° cultures), but increased the counts in all others, showing that in the first group the same bacterial types remained, while in the second group, where fermentation proceeded actively, the developed type required a richer medium for growth. Fermentation at 36° proceeded so rapidly that the three-week interval counts were not sufficiently frequent to follow the changes in bacterial population. However, at 21°, where fermentation was so much slower, the increase in the rate of gas evolution in bottles "*D*" and "*E*" was found to coincide with the highest bacterial counts.

*Types of Bacteria Encountered.*—The appearance of colonies on the Petri dishes also deserves to be mentioned. The 9° bottles, and "*A*" and "*B*" at 21°, up to the end of the 310 days had a very large variety of colonies, including some chromogenic (yellow, orange and pink colonies) and also very large surface colonies. Microscopic examination revealed a large variety of small, mostly Gram negative rods, as well as a small number of long thread-like bacteria and a few cocci. Plates of the others (the actively fermenting cultures) showed a complete absence of chromogenic bacteria and of the large surface colonies; all surface colonies were small and the imbedded colonies were mostly small and spindle-shaped. The majority of the bacteria on aerobic plates were Gram positive cocci. The anaerobic plates, in addition to these facultative organisms, contained rather heavy Gram positive rods which were not observed in the first group.

*B. Pure Culture Studies.—Anaerobic technique.*—A recent article by Scott and Brandly[5] on the use of iron filings in cultivation and study of anaerobes came to our attention, and their method was tried. Using the filing, oil seal, deep tube and hydrogen combustion (McIntosh jar) methods,

stock cultures of five anaerobes, namely: Clostridium putrificum, Cl. bifermentans, Cl. tertium, Cl. sporogenes and Cl. centrosporogenes were grown in various media. To some of the tubes methylene-blue indicator was also added. It was found that iron filings (1) produce and maintain a state of anaerobiosis (indicated by discoloration of methylene-blue to the distance of at least one centimeter above the layer of filings) and all of the five anaerobes grew well in their presence; (2) stimulate the growth of bacteria (as indicated by turbidity), apparently by neutralizing the acids formed; (3) are unsuitable for the study of physiological characteristics of bacteria, as they react with acids produced, forming gas (hydrogen) which may mislead the investigator as to the gas-forming ability of the bacteria under consideration. Thus, for example, Cl. putrificum with filings showed a large quantity of gas in dextrose, maltose, sucrose and lactose, instead of only acid as when incubated in the McIntosh jar. Other bacteria such as B. tumescens, when grown with filings showed gas in dextrose, lactose and sucrose, B. megatherium, gas in dextrose and sucrose, etc. All these organisms produced only acid in the above sugars when grown under suitable conditions without filings; and when to the above media in which acid had been produced by bacteria, filings were added gas evolution immediately took place, and the pH was restored to neutral. (4) The filings also reduce nitrates to nitrites.

In this work the McIntosh jar technic was used exclusively for the growing and the study of the anaerobic bacteria described below.

*Isolations.*—From bottle "*E*" incubated at 21°C. the bacteria were isolated at the end of the fermentation period in three different ways: from aerobic and anaerobic plates using ordinary dextrose agar, and anaerobically using Beckwith and Moser's[3] sulfite agar for $H_2S$ forming bacteria. After ten days of incubation the plates were examined under a dissecting microscope and in each series, from a dilution containing 30 to 40 colonies per plate, all well isolated single colonies were picked from a certain section of the plate and transplanted on agar slants. From an aerobic plate eight colonies were taken; two did not develop, five proved to be small, Gram positive, facultative cocci and one a facultative, very actively motile, Gram negative rod (P-10). From an anaerobic dextrose agar plate, eight more colonies were taken; three proved to be $H_2S$ formers, and five were Gram positive, slow-growing, gas-forming rods. The Beckwith and Moser's sulfite agar anaerobic plate (dilution 1 to 1000) had 47 colonies out of which 18 were $H_2S$ formers (indicated by a black halo of FeS formed around each colony). Seventeen colonies were picked from half of the plate; of these nine were $H_2S$ producing rods, one Gram positive cocci and the rest were Gram positive, gas-producing, relatively large rods.

From the preliminary examination, i.e., staining characteristics, morphology, motility, relation to oxygen, and production of hydrogen sulfide,

TA-

DESCRIPTION

| GENUS | CULTURE NUMBER | MORPHOLOGY AND STAINING CHARACTERISTICS | COLONY FORM ON AGAR | GELATIN STAB. | PEPTONE BROTH, INDOL FORMATION |
|---|---|---|---|---|---|
| Micrococus | P-1, P-2, P-3 | Spheres, 0.7 to 1.0 $\mu$ in diam. occurring singly, in pairs and short chains of 4–5 organisms. Large involution forms occasionally present. Gram positive | White, glistening, raised; margin entire; somewhat fluorescent | No liquefaction | Slight growth; indol not formed |
| Flavobacterium | P-4, P-5, P-6, P-7 | Rods, 0.5 by 1.2 to 1.8 $\mu$, occurring singly and in short chains; actively motile; spores not formed; encapsulated. Gram positive | Small, convex, smooth, circular buff colored; margin entire | Rapid stratiform liquefaction. Dark zone along the line of growth precedes liquefaction | Abundant growth; turbid; indol not formed |
| | P-10 | Rods, 0.5 by 1.2 to 1.5 microns; occurring singly; actively motile; spores not formed. Gram negative | Convex, circular, smooth, white | Slowly liquefied | Very abundant growth; indol not formed |
| Clostridium | P-8 | Rods, 0.7 by 2 to 3 $\mu$, occurring singly and in short chains; sluggishly motile; small terminal spores seldom formed. Gram positive | Raised, smooth, white, margin entire; cohesive, mucilagenous | No liquefaction | Scant growth; indol not formed |
| Clostridium | P-11, P-12, P-13 | Rods, 0.8 to 1.0 by 3 to 4.5 $\mu$, occurring singly and in chains up to ten organisms; actively motile; large, oval subterminal spores; rods swollen on sporulation. Gram positive | Raised, smooth, white; margin entire; cohesive, mucilagenous | No liquefaction | Very slight growth: indol not formed |

it was apparent that the isolated bacteria could be roughly divided into four groups: (1) Gram positive, facultative cocci; (2) small, Gram positive facultative, hydrogen sulfide producing rods; (3) relatively large, Gram positive, gas forming rods, and (4) a single Gram negative rod (P-10). Consequently three organisms were selected at random from the first group (given Numbers P-1, P-2, P-3), four from the second (P-4, P-5, P-6, P-7) and five from the third (P-8, P-9, P-11, P-12, P-13). The thirteen bacteria selected were then plated and picked twice and studied in detail. The facultative organisms were always carried in two sets: both aerobically and anaerobically; the anaerobic cultures were incubated in McIntosh jars.

The results of the pure culture studies showed that there were five distinct species as follows:

BLE 2

OF BACTERIA

| REDUCTION OF NITRATES | LITMUS MILK | FERMENTATION OF CARBOHYDRATES: GLUCOSE | MALTOSE | SUCROSE | LACTOSE | MANNITOL | LEVULOSE | GALACTOSE | INULIN | SALICIN | GLYCEROL | RELATION TO OXYGEN, SPECIAL CHARACTERISTICS, REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reduced to nitrites | Litmus reduced, no other change | × | × | × | × | − | | | | | | Facultative, grows equally rapidly under both aerobic and anaerobic conditions<br>Isolated from sea water mud |
| | | Acid production very rapid | | | | | | | | | | |
| Not reduced | Coagulated, peptonized | − | − | − | − | − | | | | | | Facultative, grows more rapidly and abundantly under aerobic conditions. Anaerobic cells considerably larger than aerobic<br>Hydrogen sulfide is produced under anaerobic conditions from sulfites but not from sulfates<br>Grows on 10% NaCl agar, but not on 15% NaCl agar<br>Optimum temperature 28 to 37° |
| Reduced to nitrogen | No change | − | − | − | − | − | | | | | | Facultative, grows better aerobically |
| Reduced to nitrites | No change | ⊗ | ⊗ | ⊗ | ⊗ | ⊗ | ⊗ | ⊗ | − | ⊗ | − | Obligate anaerobe<br>Grows exceedingly slowly on plain agar; better growth is obtained if dextrose is present and the surface is moist |
| Not reduced | Slight acid | ⊗ | ⊗ | ⊗ | ⊗ | ⊗ | ⊗ | ⊗ | ⊗ | ⊗ | − | Obligate anaerobe<br>Scant growth on plain agar; addition of sugar, low per cent of agar (about 1.0%) and moist surface favor better growth<br>The dead rods form peculiar wide shadow forms |

Species 1 P-1, P-2, P-3
Species 2 P-4, P-5, P-6, P-7
Species 3 P-10
Species 4 P-8
Species 5 P-11, P-12, P-13

the bacterium P-9 was lost.

The five species are described in table 2.

None of the species here described seem to fit into the description of any known species as given in the 1934 edition of Bergey's Manual.[3]

*C. Fermentation of Sulfite Waste Liquor by Bacteria in Pure Culture.*—Gas production from the s.w.l. by pure cultures of the isolated bacteria was next studied. In order to obtain a good comparison as to the amount

of gas formed, Durham fermentation tubes were placed on small wooden tooth-picks, thus raising their open end about five mm. from the bottom of the test tube and allowing most of the gas formed on the bottom to collect in these ubes. The gas data are, therefore, expressed as the per cent of gas in Durham tubes. The percentages in the table 3 below were obtained after seven days of incubation.

TABLE 3

GAS PRODUCTION BY PURE CULTURES IN S.W.L.

| MEDIA | PERCENTAGE OF GAS P-8 | P-11 | P-13 |
|---|---|---|---|
| 20% s.w.l. | 1% | 0.5% | 0.5% |
| " + iron filings | . | 5 | .. |
| " + agar, 2.0 gr./liter | 1 | 3 | .. |
| " + beef extract, 2.0 gr./l. | 5 | 80 | 55 |
| " + beef extract, 2.0 gr./l. + 0.5% dextrose | 5 | 65 | 90 |
| " + $KNO_3$ + $K_2HPO_4$ 0.5 gr./l. each | .. | 0.5 | .. |
| " + peptone, 5.0 gr./l. | 5 | 50 | 40 |
| " + asparagine, 1.0 gr./l. | . | 1 | 1 |

Table 3 shows that presence of nitrogenous organic material such as peptone and beef extract is essential for good gas production from s.w.l. by pure cultures of these bacteria. That it is the nitrogenous organic content and not the inorganic salts of beef extract that are of moment is demonstrated by table 4, which shows that the amount of gas formed is directly dependent on the amount of beef extract added. The beef extract used had a residue on ignition of 22.1 per cent.

TABLE 4

GAS PRODUCTION IN RELATION TO CONCENTRATION OF BEEF EXTRACT

| BEEF EXTRACT ADDED (GR./L) TO 20% S.W.L. | GAS PRODUCED BY P-11 IN PER CENT |
|---|---|
| 2.0 | 20 |
| 1.0 | 10 |
| 0.6 | 8 |
| 0.4 | 2 |
| 0.2 | 3 |
| 0.1 | 0.2 |
| 0.05 | none |
| Beef extract (two gr./l.) without s.w.l. | none |

The presence of colloidal matter has been found by many investigators to favor gas production by bacteria. We have tried adding to the fermentation tubes containing diluted s.w.l. sea mud, pulverized quartz and sand, and found that with mud, gas production was about 30 per cent of the Durham tubes, with quartz and sand about 10 per cent, while in liquor alone much smaller amounts of gas were produced.

To test the effect of s.w.l. on the growth and fermentation of well-known microörganisms the following nine were selected from the University stock and inoculated into s.w.l. containing media as summarized in table 5.

TABLE 5

EFFECT OF S.W.L. ON SEVERAL COMMON MICROÖRGANISMS

| | MEDIA | | | | | |
|---|---|---|---|---|---|---|
| ORGANISM | (1) GLUCOSE AGAR SLANT WITH 15% S.W.L. | (2) S.W.L. DILUTED WITH WATER 1 TO 5 | (3) AS MEDIA (2), BUT WITH 2 GR./L. BEEF EXTR. ADDED | (4) AS MED. (2), WITH 10 GR./L. PEPTONE AND 3 GR./L. BEEF EXTR. | (5) AS MEDIA (4) BUT WITH 1% GLUCOSE | (6) BROTH WITH 1% GLUCOSE (NO S.W.L.) |
| | PERCENTAGE OF GAS | | | | | |
| Sach. cerevisiae | G.G. | 0.2% | 0.2% | — | 10 | 55 |
| E. coli | G.G. | 1 | 4 | 10 | 15 | 40 |
| E. neopolitana | G.G. | 2 | 10 | 2 | 5 | 30 |
| E. communior | G.G. | 0.5 | — | 5 | 6 | 35 |
| E. pseudo cosscoro-dae | G.G. | 3 | 8 | 15 | 12 | 35 |
| A. aerogenes | G.G. | 0.5 | — | 4 | 5 | 35 |
| Cl. sporogenes | G. | iii | iii | iii | 30 | 40 |
| Cl. centro-sporo-genes | G.G. | iii | iii | iii | 2 | 25 |
| Cl. befermentans | G.G. | iii | iii | iii | 40 | 25 |

Symbols:

G.—growth; G.G.—good growth.

— no action.

i—the number of "i" is equal to the number of unsuccessful inoculations tried.

*Summary.*—1. Aerobic bacterial counts during fermentation of s.w.l. diluted with varying amounts of sea water, placed over marine mud and incubated at three temperatures, were made through a period of 310 days.

2. A number of anaerobic and facultative bacteria were isolated from the fermenting bottles and thirteen were studied in pure culture; they represented five different species herein described, none of which seem to fit any species given in Bergey's Manual.

3. The isolated bacteria were shown to be able to ferment s.w.l. with the evolution of gas; the addition of beef extract and peptone increased the gas production.

4. S.w.l. when present in concentrations of 15 to 20 per cent in ordinary bacteriological media, such as glucose agar and glucose broth, does not prevent but only somewhat slows down the growth and fermentation of nine common microörganisms (one yeast, five facultative and three anaerobic bacteria) tried. Alone or with beef extract or peptone s.w.l. in the above concentration is fermented by these organisms to only a limited extent.

Acknowledgment is made to the National Research Council for the Grant-in-aid to H. K. Benson of the department of chemistry, University of Washington, whereby this study was made possible.

[1] H. K. Benson and A. M. Partansky, these PROCEEDINGS, **20**, 542 (1934).

[2] T. D. Beckwith and J. R. Moser, *Jour. Bact.*, **24**, 43 (1932).

[3] D. H. Bergey, *Manual of Determinative Bacteriology*, Fourth Edition, The Williams and Wilkins Company, 1934.

[4] C. B. Lipman, Publ. No. 340, *Carnegie Inst. of Wash.*, p. 179.

[5] J. P. Scott and C. A. Brandly, *Jour. Bact.*, **26**, 1 (1933).

[6] R. S. Spray, *Jour. Lab. and Clin. Med.*, **16**, 203 (1930).

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## *IDENTIFICATION OF THE SALIVARY GLAND CHROMOSOMES IN DROSOPHILA PSEUDOÖBSCURA*

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The nuclei of the salivary gland cells of mature larvae and young pupae of Drosophila pseudoöbscura contain five chromosome strands, each attached at one of its ends to the chromocenter, and one small chromosome imbedded in the latter. Each strand represents two closely paired homologous chromosomes or chromosome limbs, the result of somatic synapsis (Heitz[1]). Individual chromosome strands can be identified by their morphological peculiarities—the distribution of the "bands," inflated areas and the shape of the free ends.

The correspondence between the chromosomes observed in the salivary gland cells and the linkage groups known in Drosophila pseudoöbscura has been established. In males two of the chromosome strands are distinctly paler and somewhat thinner than the rest. The two pale strands are the two limbs of the *X*-chromosome. The *Y*-chromosome is invisible in salivary gland cells, probably being composed of an inert material and included in the chromocenter (Painter[2]). By studying salivary chromosomes of larvae that were known on the basis of genetic data (Lancefield,[3] Koller[4] and Sturtevant unpublished), to carry inverted sections in one or the other of the chromosomes, the right and the left limbs of the *X*-chromosome, the second chromosome (carrying the Bare Smoky linkage group) and the third chromosome (carrying the *or Sc pr* linkage group) were identified.[5] The two limbs of the *X*-chromosome in salivary gland cells are of unequal length; while in spermatogonial and nerve cell metaphase plates they are similar in length. This is probably due to one of the limbs (the left one,

carrying the gene Pointed) containing more inert material than the other limb (the right one, carrying the gene short). The fourth chromosome (carrying the gene Curly) has been recently identified by studying a translocation, obtained with the aid of x-rays, in which a linkage between Curly and the *Y*-chromosome has been observed (a 4-*Y* translocation). Cytologically a piece of the middle part of the fourth chromosome has been found to be attached to the chromocenter. The fourth chromosome forms a loop-like configuration with a second chromocenter attachment in the middle part of the chromosome, or otherwise simply shows a corresponding deficiency in one of the haploid strands. The only chromosome in which

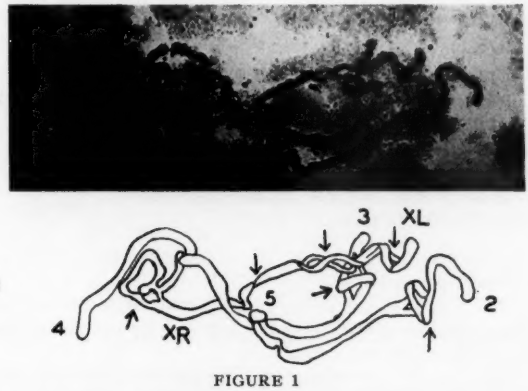


FIGURE 1

*A*.—A microphotograph of the salivary chromosome complement of $A \times B$ hybrid of Drosophila pseudoöbscura showing all interracial inversions. *B*.—An outline sketch of the above figure with each chromosome labeled (*XL*—the left limb of the *X*-chromosome, *XR*—the right limb of the *X*, 2—the second chromosome, 3—the third chromosome, 4—the fourth chromosome and 5—the fifth chromosome) and each inversion indicated by an arrow.

no genes are now available must be, therefore, the smallest autosome usually imbedded in the chromocenter. The different chromosomes are labeled in figure 1.

Salivary gland cells of the hybrid larvae from the crosses of race *A* and race *B* show one large and two very small inverted sections in the left limb of the *X*-chromosome, a rather long inversion in the right limb of the *X*-chromosome, in the second and in the third chromosomes. Such a chromosome complement of a hybrid larva is shown in figure 1. These inversions may be safely considered as distinctive properties of the two races, since they were found in all of more than twenty different interracial crosses

which involved various strains of race *A* and race *B* coming from diverse parts of the distribution area of each race. These races were of different "strength," and had different types of *Y*-chromosomes (Dobzhansky and Boche,[6] Dobzhansky[7]).

The details of chromosome morphology and chromosome differences between the two races of Drosophila pseudoöbscura is now in press elsewhere.[5] The present identification of the salivary chromosome which carries the gene *Cy*, however, has completed the identification of the salivary chromosomes which correspond to each of the four linkage groups so far known in Drosophila pseudoöbscura. An effort to construct a cytological map of the chromosomes of this species which will locate the principal genes in each chromosome with reference to various sets of characteristic "bands" is now in progress.

[1] Heitz, *Zeit. Zellf. u. mikr. Anat.*, **20**, 237–287 (1933).
[2] Painter, *Genetics*, **19**, 175–188 (1934).
[3] Lancefield, *Zeit. ind. Abst. Ver.*, **53**, 287–317 (1929).
[4] Koller, *Ibid.*, **60**, 137–151 (1932).
[5] Tan, *Genetics*, in press.
[6] Dobzhansky and Boche, *Biol. Zbl.*, **53**, 314–330 (1933).
[7] Dobzhansky, *Genetics*, in press.

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## *THE BEHAVIOR OF UNSTABLE GENIC LOCI—AN HYPOTHESIS*

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During the last years the writer has studied the origin of mosaic spots on the body surface of *Drosophila melanogaster*. The cause of mosaic formation was found to be the occurrence of somatic crossing-over and subsequent segregation.[1] Based on these findings a working hypothesis has been built up to explain the curious behavior of "eversporting" eye colors in *Drosophila*. The speculative character of the following pages is recognized but the hypothesis seems to be able to explain facts hitherto not understood and may be subjected to further tests.

1. Gowen and Gay[2] describe the behavior of three mutations, causing reddish white mottled eye color in *D. melanogaster* which originated from x-rayed males carrying the normal, red-eyed allel at the white-locus in the *X*-chromosome. These somatically "eversporting" characters appeared first in single individuals which as a result of radiation were found to possess complexly broken and translocated chromosomes different in each of the three cases. This mottling is a property of the white-locus; the di-

rection of color change being from red to white. The symbol (*mo*) is here used for the three different determiners of mottling alike. Assuming that the "gene" for mottling consists of a normally located allel "white" (*w*) and a normal allel ($w^+$) laterally attached to the chromosome next to the *w*-locus (Fig. 1*a*) and assuming that somatic crossing-over occurs frequently between the attached part of a chromatid and the homologous normally located part of the sister chromatid (Fig. 1*b*), the result will be two new chromosomes, one possessing a lateral duplication of the whole left end, the other deficient for the whole left end (Fig. 1*c*). Cell patches in the eyes of males containing the duplicated chromosome should be white; cells containing the chromosome which lacks the left end die. The red regions of the eye therefore would have the original chromosomal constitu-

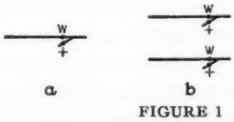


FIGURE 1

(*a*) The assumed constitution of a chromosome fragment containing white-mottled.

(*b*) The chromosome split into the daughter chromatids, before crossing-over has occurred.

(*c*) The two chromosomes resulting from crossing-over between one main chromatid and the lateral translocation of its sister chromatid.[3]

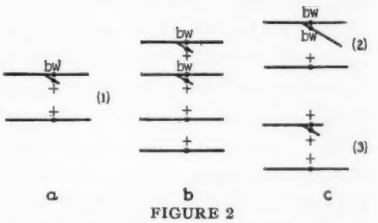


FIGURE 2

(*a*) The assumed constitution of a *Pm*/+ zygote.

(*b*) The chromosomes split into the daughter chromatids before crossing-over has occurred.

(*c*) The two daughter-cells resulting from segregation after crossing-over between one main chromatid and the lateral translocation of its sister chromatid. (1), (2) and (3) refer to the text.

tion; the white regions result from somatic crossing-over. That the red regions are smooth, and the white regions rough with deep spaces between the ommatidia is in agreement with the hypothesis. The character of the white regions would result from the hyperploid condition of its cells and possibly from the lethal hypoploid cells. Females of the constitution *mo*/*w* also show mottling, but the white regions are not rough due to the less striking phenotypic effect of a hyperploid condition in females.

2. Muller, Van Atta, Glass, Schultz and Dobzhansky[4] and others have described a number of "eversporting" dominant eye colors produced in x-rayed individuals of *D. melanogaster*. In most of these cases it was shown that a break with subsequent translocations or inversions had occurred at or very near the second chromosome locus "brown." Brown is a non-mottling recessive eye color. The induced change at the brown-locus (the

treated flies were originally wild type) produces a dominant effect on the eye color. In the following discussion the symbol *Pm* (Plum) is used to designate these dominant determiners. A *Pm*/+ individual shows an eye mottling of darker and lighter patches of brownish purple color. The extent and number of the dark areas vary in different individuals: in some appearing as smaller spots on a lighter ground; in others forming the background. The testis sheath, transparent in homozygous brown flies, is uniformly pigmented in *Pm*/+ as well as in wild flies. Assuming that the *Pm*-"gene" consists of a normally located allel "brown" (*bw*) and a normal allel of brown (+) laterally attached next to the *bw*-locus (*bw*,+) (Fig. 2*a*), if somatic crossing-over occurs as assumed in the white-mottling, one would expect three types of cell areas (Fig. 2*a*, *c*):1, (*bw*,+)/+, 2, (*bw*,*bw*) dupl./+ and 3, (+,+) def./+. Type (3) would be nearly lethal, since Pale deficiency which covers the same region is lethal. If the normal, $+^{bw}$ containing chromosome has recessive genes in its extreme right end, then surviving cells with the constitution (3) should allow the phenotypic expression of such genes, since the homologous chromosome is deficient for them. This would account for the small spots (generally single bristles) of the gene minus. What color would cells of the constitutions (1) and (2) produce? Normally one wild-type allel of *bw* is completely dominant over one or two recessive *bw*. If the homogeneously pigmented testis sheath in *Pm*/+ individuals is composed of the supposed areas (1) and (2), as becomes probable from the description of mottled sheaths in other *Pm* combinations, the dominance of $+^{bw}$ in these areas will be apparent. However, the eye-color appearance in the *Pm*/+ flies will have to be understood as a semidominant effect of the peculiar configurations at the *Pm*-locus. It is assumed that the areas (2) exhibit the lighter color in the mottled eye. The darker areas would be represented by the constitution (1). This dark color which departs only slightly from the normal wild color is probably a product of the constitution (1) under the influence of the neighboring areas (2).

The hypothesis concerning the *Pm*-variegation can be tested by an analysis of other combinations of *Pm* with changes at the *bw*-locus. A few will be described here. *Pm*/*bw* shows an almost homogeneous brown eye color with some ill-defined mottling. The testes, however, are distinctly mottled. Considering only the somatic constitutions (1) and (2)—after substitution of *bw* in the not-*Pm*-chromosome—we obtain: 1, (*bw*,+)/(bw), 2, (*bw*,*bw*) dupl./*bw*. Areas (2) would be brown in eye color and transparent in testes sheaths, areas (1) would be less extreme *bw* in eye color and pigmented in testes sheaths.

Over 90% of the homozygous *Pm*/*Pm* individuals are non-viable. Those which develop far enough possess a nearly homogeneous brown eye color. Somatic crossing-over in *Pm*/*Pm* would lead to (1) (*bw*, *bw*) dupl./(*bw*,+)

cells, if the crossing-over process had occurred only within the chromatids of one of the two *Pm* chromosomes; it would lead to (2) (*bw,bw*) dupl./(*bw,bw*) dupl. if it had occurred in both homologues. The areas (2) will appear brown, the areas (1) probably brownish too (similar or browner than the areas (1) discussed in the last paragraph). The nearly lethal cells with the constitution (3) (+,+) def./(*bw*,+) will give very small, non-brown spots, which have been found. The extremely low viability of the *Pm/Pm* flies as well as their aberrant phenotype would be due to the presence of a homozygous duplication in all areas (2). Addition of the known Pale duplication (which represents the *bw*-end of the chromosome II translocated to III) would decrease the viability of *Pm/Pm* still more by giving rise to cells containing the duplication three times. *Pm/Pm* Pale dupl. flies indeed have never survived. (Cf. also footnote 8.) In some *Pm*-allels at least, the light-locus is involved also. However, the data presented so far seem too inadequate to be included in this discussion.

3. The presence of an extra *Y*-chromosome (*XYY* in males, *XXY* in females and intersexes) suppresses completely or nearly completely the "unstability" of both the white-mottled and the Plum locus. In Plum an extra *Y*-chromosome suppresses also the dominance effect as well as the instability for minus. These curious influences of the *Y*-chromosome can be interpreted uniformly as a suppression of somatic crossing-over at the eversporting locus. The subsequent non-appearance of (*w,w*) dupl. (Fig. 1*c*) accounts for the absence of white eye patches and the non-appearance of (*bw,bw*) dupl. (Fig. 2*c*(2)) for the absence of the dominant effect of *Pm*. The corresponding non-appearance of ($+^{bw}+^{bw}$) def. (Fig. 2*c*(3)) in the case of *Pm* accounts for the stability for minus. The very good viability of *Pm/Pm* extra *Y* individuals which are nearly normal in eye color, is also explained by the non-appearance of (*bw,bw*)dupl./(*bw,bw*) dupl. areas.

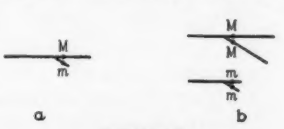


FIGURE 3

If (*a*) the double gene (*M*, *m*) will produce the phenotype of "*m*," then (*b*) crossing-over will lead to a chromosome carrying only *M* and exhibiting only this phenotype.

Discussion as to how the supposed suppression of crossing-over by the *Y*-chromosome is brought about will be omitted; however it might be pointed out that all experimentally induced eversporting "genes" seem to be located very near the spindle-fibre as a consequence of chromosome rearrangements. This brings them into a topographical neighborhood with the chromocenter. In the case of white-mottled, the suppression of eversporting, assumed to be the suppression of crossing-over, is also brought about by the addition of an extra *X*-chromosome (in *mo*-1, triploids are not mottled, while intersexes are mottled). The effect therefore does not seem to be specific for the *Y*-chromosome.

4. Do the so-called "mutable" genes, as described in numerous organisms, also fall under the suggested hypothesis? The change in most "mutable" genes follows the direction from the "recessive mutant" to the "dominant wild-type" state. If the hypothesis of a semidominant effect of an otherwise recessive gene (*m*) in a "double" gene combination (*m*,*m*), as discussed to explain the appearance of Plum (Fig. 2*c*(2)), is extended to the idea of a recessive effect equal to (*m*) of a double gene of the constitution (*M*,*m*) then, a constitution having the normal allel (*M*) lying at its normal locus with the allel (*m*) (Fig. 3*a*) laterally attached would produce the phenotype *m*. By crossing-over a condition like figure 3*b* (upper chromosome) will originate with the phenotypic effect *M*. Such a scheme satisfies the direction of "mutation" phenotype *m* ⟶ phenotype *M*. An explanation of "mutation," in "mutable" genes by a mechanism like crossing-over makes understandable the high correlation in *Drosophila virilis* (Demerec)[5] between the "mutations" of the gene reddish-$\alpha$ and crossing-over in its immediate neighborhood, as well as the striking effect of the type of allel present in the homologous locus of the mutable reddish-$\alpha$: Thus any non-mutable allel in combination with re-$\alpha$ allows re-$\alpha$ to mutate; in homozygous re-$\alpha$/re-$\alpha$, however, no mutations occur. If the mutable re-$\alpha$ is represented by a translocation in contrast to all the other of its allels, then it is obvious that the conditions for crossing-over are different in the combination homozygous for the translocation from all others in which the translocation is present in heterozygous condition.[6] Finally, it may be pointed out that the types of arrangements given in figure 4 give a model for the $\gamma$- and $\beta$-allels of mutable genes (Demerec)[7] with their different kinds and frequencies of mutability. Probably not all of the many types of mutable genes lend themselves to an explanation of the type suggested.

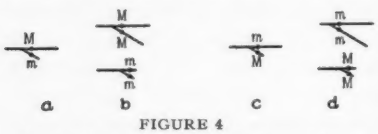


FIGURE 4

(*a*) Model of a *frequently* "mutating" allel: The phenotype of (*a*) is assumed to be "*m*." Crossing-over leads to (*b*) upper part, with the "mutated" phenotype "*M*." The lower configuration in (*b*) is regarded to be lethal.

(*c*) Model of a *rarely* "mutating" allel: The phenotype of (*c*) is again assumed to be "*m*." Crossing-over leads to (*d*) upper part, with the unchanged phenotype "*m*." The lower configuration in (*d*) is again assumed to be lethal. Other types of crossing-over can change (*a*) into (*c*) and vice versa.

It is probable that division of the dupl.-chromosomes (upper configuration in (*b*) and (*d*)) can lead to single thread chromosomes without duplications. The position of the spindle-fibre—near the "mutable" locus in the white-mottled and Plum stocks, but distant in other cases of mutable genes—may be of significance.

*Conclusions.*—The hypothesis discussed in this paper to account for the behavior of unstable loci is based on the following assumption: The unstable locus is a composite of a gene in its normal locus (in respect to some

neighboring loci) and of a mutant allel of this gene laterally attached next to it. To account for the unstability the occurrence of somatic crossing-over between the attached part of a chromatid and the homologous normally located part of the sister chromatid is assumed. To account for the dominant effect of many eversporting loci a position effect is assumed according to which two mutant allels located together exert a dominant effect in contrast to their recessive effect if localized singly. Also, the compound locus of the normal and the mutant allel is assumed to exert an effect different from the effect produced by a normal and a mutant allel which are located in different chromosomes.

These assumptions on dominance might be regarded as very unorthodox. The dominance relation of two allels in directly neighboring positions, however, cannot be predicted from the known relation of these allels in their normal, separated locations. The standard case of a position effect, the Bar-case, is just one in which the effect of two allels is increased if together in one chromosome ($BB/+$) as compared to their distribution over two chromosomes ($B/B$). The interpretation of the "simple" Bar-gene might be effected by our assumption also. Dobzhansky's[9] finding of a new change at the Bar-locus, dominant in the first, but recessive in all later generations might be regarded as the formation of a compound of a normal and a Bar-allel in the first generation, giving a dominant effect together and a subsequent loss (by crossing-over?) of the normal allel, leaving only the baroid gene with its recessive effect.

No attempt has been made either to discuss all consequences or all advantages and all difficulties of the system of hypotheses, nor is it believed that it will survive in a totally unchanged form.[10] However, its formal success at present in explaining some apparently very strange facts might make it of further use.

[1] Two abstracts have already been published: *Amer. Nat.*, **68**, 164–165 (1934); *Ibid.*, **69**, 81–82 (1935).

[2] *Proc. Nat. Acad. Sci.*, **19**, 122–126 (1933); *Genetics*, **19**, 189–208 (1934); similar cases have been reported by Muller, *Jour. Genetics*, **22**, 299–334 (1930).

[3] The diagrams are not intended to suggest that the chromosomes in mitosis are *visibly* branched. Both branches might be included within the stained substance of a normal appearing chromosome.

[4] Muller, *Jour. Genet.*, **22**, 299–334 (1930); Van Atta, *Genetics*, **17**, 637–659 (1932); Glass, *Amer. Nat.*, **68**, 107–114 (1934); *Jour. Genet.*, **28**, 69–112 (1934); Schultz a. Dobzhansky, *Genetics*, **19**, 334–364 (1934).

[5] *Genetics*, **13**, 359–388 (1928).

[6] R. A. Emerson, *Genetics*, **14**, 488–511 (1929) has observed similar though less extreme effects of different types of allels on the mutability of unstable loci in corn.

[7] *Proc. Nat. Acad. Sci.*, **15**, 870–876 (1929).

[8] Somatic crossing-over is known to occur frequently between chromatids of homologous chromosomes, and not only sister chromatids. Such a type of crossing-over might play a rôle in the eversporting behavior also. (If our conception of the mutability of

reddish-$\alpha$ should prove true, germinal crossing-over between homologous chromosomes would occur there.)

[9] *Genetics*, **17**, 369–392 (1932).

[10] A comparison with earlier hypotheses of other authors has also been omitted.

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## *POLLEN-TUBE GROWTH OF A TRANSLOCATION OF THE 1·2 CHROMOSOME IN DATURA*

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The present paper has to do primarily with the effect upon pollen-tube growth of extra chromosomal material brought about through radiation treatment. In this particular case the pollen tubes were exposed to radon during their passage through the style, according to the method described in an earlier publication.[2] Due to the relatively greater distance of the ovary from the radon it is probable that the induced changes were brought about in the sperm cells within the pollen tubes as they grew through the field of most intense activity induced by the tube of radon which was closely applied to the style. In one of the four locules of the capsule which matured following this treatment there were no good seeds which germinated and but one each in the remaining three locules. Of the three plants which grew from these three seeds, one was normal in appearance and had normal offspring. A second was recorded as off-type and was found to be a chromosomal deficiency. The third, 27377(1), was heterozygous for a segmental interchange as well as heterozygous for the simple translocation with which we are here concerned.

The cytology of this translocation has been described in an earlier paper[3] and need not be discussed in detail here. It is sufficient to say that a break was induced in the 1·2 chromosome in the region of the spindle fibre insertion. The ·2 half was translocated to the ·11 end of the 11·12 chromosome to form a new large 2·11·12 chromosome and the ·1 half was left as a free fragment. That the 1·2 chromosome was involved in some kind of chromosomal abnormality in the parent plant 27377(1) was shown by its offspring in which out of a total of 28 plants there were 11 recognized as resembling the secondary chromosomal type $2n+2\cdot2$, called also "Sugarloaf" from the shape of its capsules. One of these plants when selfed gave offspring with 7 extreme Sugarloafs, 30 weakly Sugarloafs like the parent and 19 plants normal in appearance. In the extreme Sugarloafs we had

gotten rid of the ·1 free fragment and of the normal 11·12 chromosomes while we had rendered the plants homozygous for the 2·11·12 chromosome. These plants, therefore, breed true and have been cultivated for eight generations as Prime Type 5.[4] In a similar way a race (PT 6) was extracted in which the 1·2 and the 11·12 chromosomes were eliminated and homozygosity secured for both the ·1 free fragment and for the 2·11·12 chromosome. Homozygous PT 5 plants[5] cannot be distinguished in external appearance from the secondary $2n+2\cdot2$ type as might be expected since both contain two extra ·2 halves of the 1·2 chromosome. The $2n+2\cdot2$ type, however, cannot breed true since it forms two types of gametophytes—$n$ and $n+2\cdot2$. The homozygous PT 5 race breeds true because all the gametophytes are $n+2\cdot\cdot$ and alike.[6] The gametophytes of PT 5 have half the unbalance of those which carry extra chromosomal material in $2n+2\cdot2$, but the unbalance in the sporophytes is the same—two extra ·2 halves. Due to this unbalance the back cross of a heterozygous PT 5 to normals gives less than 50 per cent of the offspring with extra ·2 material. In one series the female backcross produced 19 plants of the $2n+2\cdot\cdot$ type out of 73 offspring while the male backcross produced the same number of heterozygous types out of 67 offspring.

The effect of the added ·2 half on the rate of pollen-tube growth in the $2n+(2\cdot\cdot)_2$ race is very significant, as was shown by a series of tests in the summer of 1929. The distance of penetration of the $n$ and $n+2\cdot\cdot$ pollen tubes, from $2n$ Line 1 controls and $2n+(2\cdot\cdot)_2$ plants, respectively, were compared at the end of a given time under uniform conditions. All tests were made on styles of Line 1 plants.

For purposes of comparison, an index of pollen-tube length was calculated as previously described[7] by averaging the distances reached by (*a*) the longest pollen tube, (*b*) the 5th, (*c*) the 10th, (*d*) the 20th tube and (*e*) the mode of distribution. The first four of these values could be accurately determined, while the position of (*e*) the mode which would require pollen-tube counts could be made approximate by inspection. The accuracy of the observation from inspection is very high, and since these five values were averaged to give us our index of length in the comparison, any slight errors made in (*e*) are ultimately divided by five. The values (*a*) to (*e*) are usually very closely proportional to each other so that a large enough series of any one of these values, such as (*a*) or (*e*) alone, would serve as a good index of comparison. A study of the deviations of each measurement from the mean of its class showed that the data of (*e*) were on the whole, probably as reliable as the rest of the observations.

The following is a summary of the data which were obtained from tests of the pollen of $2n+(2\cdot\cdot)_2$ plants and normal $2n$ controls.

Mean length—index of 55—$2n$ control plants (12 hours)

$32.37 \pm 0.16$ mm. $\sigma \pm 1.17$ mm.

Mean length—index of 35—$2n+(2\cdot\cdot)_2$ plants (12 hours)
28.17 ± 0.17 mm. $\sigma$ ± 1.46 mm.
Observed Difference = 4.20 ± 0.23 mm.
Difference /Error of Difference = 18.3

Translated into percentage with $2n$ controls taken as 100, the $n+2\cdot\cdot$ pollen tubes were found to grow 87.03 ± 0.95 per cent as far in 12 hours as those of the $2n$ controls. In $2n$ controls, $\sigma$ = 5.29 per cent and in $2n+(2\cdot\cdot)_2$, $\sigma$ = 5.18 per cent. A distribution curve for pollen tubes of $2n$ plants is shown in figure 1*A*; those of $2n+(2\cdot\cdot)_2$would be similar and 87 per cent as long, with more ungerminated pollen.

We may assume that pollen-tube growth in the $2n+(2\cdot\cdot)_2$ race is uniform, giving no acceleration or retardation in growth rate, since we have found this to be true in control Line 1 plants.[7,8] It is also true of *Datura meteloides* and of a cross *Datura stramonium* × *Datura meteloides*.[8] Thus the difference in *growth-rate* of pollen tubes in the two races is probably directly proportional to the above difference observed in comparable 12- and 16-hour tests, provided further, that we may assume an equal promptness in the germination of the two kinds of pollen, which is indicated by all of our indirect evidence.

If the depression in pollen-tube growth-rate when a single ·2 fragment is added is 87 per cent, we might expect to obtain a depression of about 74 per cent when twice as much extra material is added as is the case in the 2·2 extra chromosome of the secondary $2n+2\cdot2$ types. Consequently we made a study of 34 test slides of pollen from $2n+2\cdot2$ plants in 1929, and of another set in 1931.

Figure 1*B* shows the distribution of pollen-tube ends in one of these $2n+2\cdot2$ tests. We have placed above it (*A*) a test of the pollen of a $2n$ plant made at the same time for comparison. As we have previously pointed out,[9] the group at the right contains the $n$ pollen tubes, the second group near the middle contains very largely the $n + 2\cdot2$ tubes, and the group of burst-pollen tubes plotted below the datum line is mostly composed of tubes containing the $n + 1\cdot2$ primary chromosome. The $n - 1\cdot2 + 2\cdot2$ to be expected in numbers approximately equal to the $n + 1\cdot2$ give shriveled pollen grains more or less devoid of protoplasmic contents and were not included in the diagram.

In evaluating the tests of the pollen of $2n + 2\cdot2$ plants we can utilize only one set of measurements, that of the modes of the two groups. The composite length index used in the former measurements is not applicable for the $n + 2\cdot2$ group of pollen tubes, since it is obviously impossible to identify the longest, 5th, 10th and 20th tubes belonging to this second group, and it is impossible to obtain $n + 2\cdot2$ pollen by itself for direct comparison.

The majority of our first 34 tests were counted under high magnification and plotted for 0.66-mm. intervals. The modes in some could be measured from inspection with the aid of the mechanical stage. The distance of the second mode was expressed as its percentage of the distance of the first mode. The 34 test slides gave the second mode at an average of 61.8 ± 0.81 per cent of the length of the first mode, with $\sigma = \pm 7.11$ per cent. It should be kept in mind, however, that pollen-tube growth may react in much the same way as morphological characters upon which a double dose of genes or of extra chromosomal material frequently exerts more than twice the effect of a single dose.

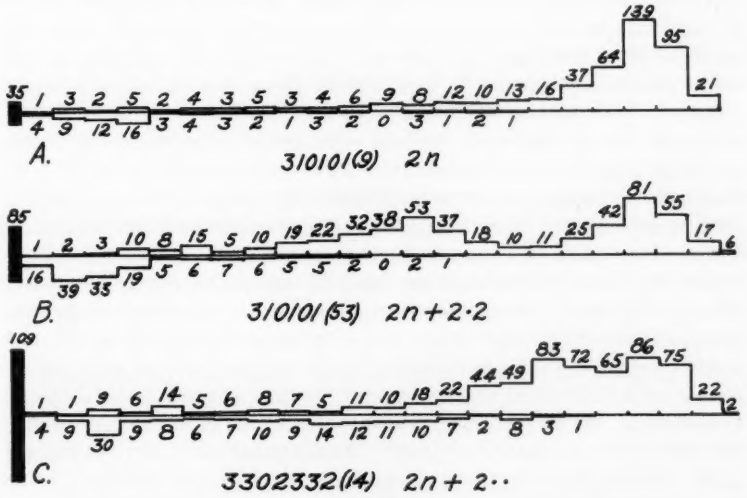


FIGURE 1

Diagrams of distribution of pollen tubes after 15 hours at 19°C. Heavy vertical bar represents ungerminated grains on stigma at left. Ends of normal pollen tubes are plotted above, those with burst tubes below the datum line, in 2-mm. intervals. *A*, Pollen of $2n$. *B*, Pollen of $2n + 2{\cdot}2$. *C*, Pollen of $2n + 2\cdot\cdot$.

This value (61.8 for the mode of the $n+2{\cdot}2$) tubes is less than the expected 74 per cent. However, when we separated this series of 34 tests (made at intervals over a period of 6 weeks) according to dates, we found considerable differences. On July 22 when pollen was employed from three $2n+2{\cdot}2$ plants, six tests showed the second mode to be at an average distance from the stigma equivalent to 56.83 per cent of the first mode ($\sigma$ = 4.08); a second set of 5 tests made on August 6 with the pollen of three plants gave an average of 55.0 per cent ($\sigma$ = 8.34); a third set of 14 tests made August 14 with pollen of 6 plants gave an average of 66.8 per cent ($\sigma$ =

4.98); a fourth set of six tests with the pollen of two plants on August 29 gave 60.56 ($\sigma$ = 4.90); and a final set of four tests on September 3 with pollen of the same $2n+2\cdot2$ plant gave the second mode at a distance of 59.12 per cent of that of the first mode ($\sigma$ = 2.11). Thus it appears that the condition of the plant at time of making the test during the growing season has considerable influence in the relative growth rates of the $n+2\cdot2$ pollen tubes.

In 1931, we made another large series of tests of the pollen of $2n+2\cdot2$ plants using the more favorable times of testing and selected 49 in which the modes stood out distinctly enough to be measured and recorded by means of the vernier of a mechanical stage. These gave a mean for the second mode at 65.02 per cent $\pm$ 0.22 ($\sigma$ = 5.68). Since this value still fell far short of the expected 74 per cent, we decided to investigate the pollen of plants heterozygous for the translocation.

The study of tests of the pollen of these heterozygotes is relatively difficult. Of course, here again, we can only compare the modes, and these are closer together and less distinctly marked, as shown in figure 1*C*. We found, for example, that most tests made after 10 or 12 hours of pollen-tube growth do not have the modes sufficiently separated to make them easily recognizable by inspection. Our most useful tests were the 15–18 hour tests. Some of the tests were actually counted, others were measured with the mechanical stage, and about a third of our tests were so abnormal with so many burst tubes or the modes so merged and confused, that no distinct modes could be recognized. From 50 selected tests made over a 4-year period in which the modes were distinct enough to be definitely recognizable, we obtained the value for the second mode as 82.9 $\pm$ 0.39 per cent of the first mode ($\sigma$ = 4.09). This value is very nearly at the mid-point between the $n+2\cdot2$ mode from the series of 49 tests and the $n$ mode. If we compare it with the 34 tests of 1929 it is higher than the mid-point, and it is distinctly lower than the value 87 per cent obtained when we compared 35 tests of pollen homozygous for PT 5 with the pollen of $2n$ plants.

It seems likely that both the $n+2\cdot\cdot$ pollen tubes and the $n+2\cdot2$ pollen tubes when present among $n$ pollen tubes and behind them, grow a little slower than they would if they were growing alone. Our experiments lend support to the former conclusion, for the latter conclusion a test is at present impossible.

That the extra 2·2 chromosome is pollen-transmitted has been demonstrated by a number of experiments.[9] In 1930 we made additional $2n+2\cdot2$ male crosses which served the added purpose of rendering plants with certain genes affecting pollen-tube growth heterozygous for this extra chromosome. In all of these cases we were successful in transmitting the 2·2 chromosome by restricting the pollen used in the cross to about 400–600 grains, and by making the pollination during a favorable time in the

growing season. The difficulty in obtaining pollen-transmission of the extra 2·2 chromosome is usually not in the inability of the $n+2\cdot2$ pollen tubes to grow through the style, but to the fact that the $n$ pollen tubes, if they are too abundant, tend to monopolize all of the ovules. When the later-arriving $n+2\cdot2$ pollen tubes reach the ovary, nearly all of the ovules are already fertilized.

With restricted pollinations the slower $n+2\cdot2$ pollen tubes have a better chance, and even then they fertilize mostly the ovules of the lower half of the ovary.[10] Thus, one of our restricted 2·2 pollinations gave, from the upper half, only one $2n+2\cdot2$ plant in 32 recorded and from the lower half 19 of the $2n+2\cdot2$ plants out of 52 plants recorded. This is 3 per cent of the $2n+2\cdot2$ type from the upper half and 38.7 per cent from the lower half of the capsule. As a control for these figures may be given data on an earlier experiment in which pollen of $2n+2\cdot2$ plants was used in crosses with normal $2n$ individuals without limitation of the amount of pollen applied to the stigmas. Out of a total of 193 offspring only four were $2n+2\cdot2$ types. This is a pollen transmission of 2.1 per cent.

It is thus obvious that the ·2 translocated fragment which is equivalent to half of the 2·2 chromosome is much more easily transmitted through the pollen as an extra than is the 2·2 chromosome. The reduction in rate of growth of pollen tubes due to extra chromosomal material is at least roughly proportional to the amount, as well as dependent upon the kind of, extra material present.

[1] This coöperative investigation is one of several made possible by grants to J. T. Buchholz from the National Research Council, Committee on Effects of Radiation on Living Organisms. Mr. L. F. Williams, who served as assistant during the period of the grants, is responsible for some of the tests and made some of the counts and measurements of pollen tubes.

[2] Buchholz, J. T., and Blakeslee, A. F., *Jour. Hered.*, **21**, 119–129 (1930).

[3] Bergner, A. D., and Blakeslee, A. F., *Bull. Torrey Bot. Club*, **61**, 197–209 (1934).

[4] Bergner, A. D., Satina, S., and Blakeslee, A. F., these PROCEEDINGS, **19**, 103–115 (1933); Blakeslee, A. F., Bergner, A. D., and Avery, A. G., these PROCEEDINGS, **19**, 115–122 (1933).

[5] Blakeslee, A. F., *Jour. Hered.*, **25**, 80–108 (1934).

[6] Two dots following the end figure is our conventional method of representing a translocated fragment without indicating the host chromosome. A single dot in front of the figure in such a formula as $2n + \cdot1$, would represent an extra free fragment.

[7] Buchholz, J. T., and Blakeslee, A. F., *Amer. Jour. Bot.*, **14**, 358–369 (1927); Buchholz, J. T., and Blakeslee, A. F., *Amer. Jour. Bot.*, **19**, 604–625 (1932).

[8] Buchholz, J. T., and Blakeslee, A. F., *Mem. Hort. Soc. N. Y.*, **3**, 245–260 (1927).

[9] Buchholz, J. T., and Blakeslee, A. F., these PROCEEDINGS, **16**, 190–195 (1930).

[10] Buchholz, J. T., and Blakeslee, A. F., *Bot. Gaz.*, **90**, 366–383 (1930).

# INSECTIVORA FROM THE SESPE UPPERMOST EOCENE, CALIFORNIA

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*Introduction.*—The insectivores now known from the uppermost Eocene stage of the Sespe (Locality 150) comprise three distinct types. The most abundant form represented at this locality is evidently related to the erinaceid genus *Proterix* from the Oreodon beds of the White River Oligocene. Associated with this type is a second genus, presumably of erinaceid affinities but known principally by an incomplete ramus of the mandible. The third genus is a member of the Leptictidae. Curiously enough, this was the first insectivore to be recognized in the fauna from Locality 150, but the type remains at present the least known of any of these forms.

## Proterixoides davisi, n. gen. and n. sp.

*Type Specimen.*—Maxillary fragment with $P\underline{3}$–$M\underline{2}$, No. 1673 Calif. Inst. Tech. Vert. Pale. Coll., plate 1, figure 1.

*Paratype.*—Ramus with $I\overline{2}$, $P\overline{3}$–$M\overline{3}$, No. 1676 C.I.T., plate 1, figures 4, 4*a*.

*Referred Specimens.*—Maxillary fragments with upper teeth, Nos. 1674, 1675 (plate 1, figure 3), 1356 and many fragments of rami with teeth including No. 1677 (plate 1, figure 2) and No. 1678 C.I.T.

*Locality.*—Brea Canyon section, Sespe deposits north of Simi Valley; Locality 150 C.I.T. Vert. Pale. Uppermost Eocene.

*Generic and Specific Characters.*—Dentition $\frac{?}{3}\ \frac{?}{1}\ \frac{?}{\overline{3}?}\ \frac{3}{3}$. $P\underline{3}$ larger than in *Proterix*, with internal cusp anterior in position and with shelf behind. Small cusp at anterior base of principal cusp. $P\underline{4}$ not molariform, but showing tendency to acquire molar crown pattern. Principal outer cusp with small posterior style and anterior basal cusp. Postero-internal portion of crown suggests incipient development of hypocone or pseudhypocone. $M\underline{1}$ broader anteroposteriorly than in *Ictops*, less so than in *Proterix*. Anterior molars wider transversely than in *Proterix*, particularly $M\underline{2}$. Hypocone and metaconule well developed. $M\underline{3}$ with antero-intermediate cusp; crown more reduced than in *Ictops*, narrower anteroposteriorly and wider transversely than in *Proterix*. $I\overline{2}$ proclivous and larger than adjacent incisors. $P\overline{2}$ apparently two-rooted. $P\overline{4}$ short, relatively wide, with heel reduced and possessing small median cusp. Paraconid progressively smaller in first to third molar, inclusive. $M\overline{3}$ slightly longer

than $M\overline{2}$, but narrower. Hypoconulid in $M\overline{3}$ well developed and situated closer to entoconid than to hypoconid. This species is named for Dr. E. F. Davis, Chief Geologist of the Shell Company, California.

*Comparisons.*—In front of $P\underline{3}$ are two alveoli in anteroposterior alignment, with the posterior socket larger than the anterior one. $I$ is a noticeably larger tooth than in *Proterix* and was evidently broader internally in an anteroposterior direction. $P\underline{4}$ in *Proterixoides* was apparently wider transversely and, relative to this width, narrower anteroposteriorly than in *Proterix*. Because of the incompleteness of this tooth in the Oligocene genus it is impossible to determine certainly whether two distinct outer cusps were present as in *Ictops* or whether a single cusp and a posterior style were present as in the Sespe type. It should be recalled that while two external roots are present in $P\underline{4}$ of *Erinaceus*, the crown possesses a single large external cusp and a small posterior wing or blade. The preserved inner lobe of the tooth in *Proterix* shows the presence of a hypocone, but this cusp is not so large as that in the molar teeth. In *Proterixoides* the beginnings of a hypocone or pseudhypocone can be discerned in several specimens where the cusp shows various degrees of distinctness. Thus, when the fourth premolar in the type specimen, No. 1673, is viewed from the postero-internal side a slight vertical furrow is evident on this surface of the protocone and a portion of the postero-external wing of the protocone is swollen. On the other hand, the origin of the hypocone as a cingular cusp is best shown in No. 1674. Here the vertical furrow is in even greater evidence and the postero-internal cingulum becomes noticeably prominent.

The types of premolars represented by $P\underline{3}$ and $P\underline{4}$ show some resemblance to comparable teeth in *Tupaia*, but several differences are likewise apparent.

In the molars of *Proterixoides* the outer cusps are conic and are situated not so close to the outer margin of the tooth-crown as in *Proterix*. In addition to the metaconule an enlargement of a portion of the antero-external wing of the protocone presumably denotes the position of an anterior intermediate cuspule. The latter is not in evidence in the Oligocene form. In $M\underline{3}$ particularly, a cuspule having this position is clearly seen in *Proterixoides* (No. 1356 in which the crown is practically unworn). This specimen likewise shows a posterior intermediate cuspule.

$M\underline{1}$ and $M\underline{2}$ are broader anteroposteriorly in *Proterixoides* and *Proterix* than in *Ictops* and *Mesodectes*, particularly internally where the larger size of the hypocone adds to the anteroposterior diameter of the tooth. In *Proterixoides*, as in *Proterix*, a noticeable change in course of outer border of the cheek-tooth series occurs at a point between $M\underline{1}$ and $M\underline{2}$. This is not so apparent in *Ictops* or in *Mesodectes*, where the outer border of $M\underline{2}$ is still nearly horizontal and the deflection inward becomes more striking along the outer border of $M\underline{3}$.

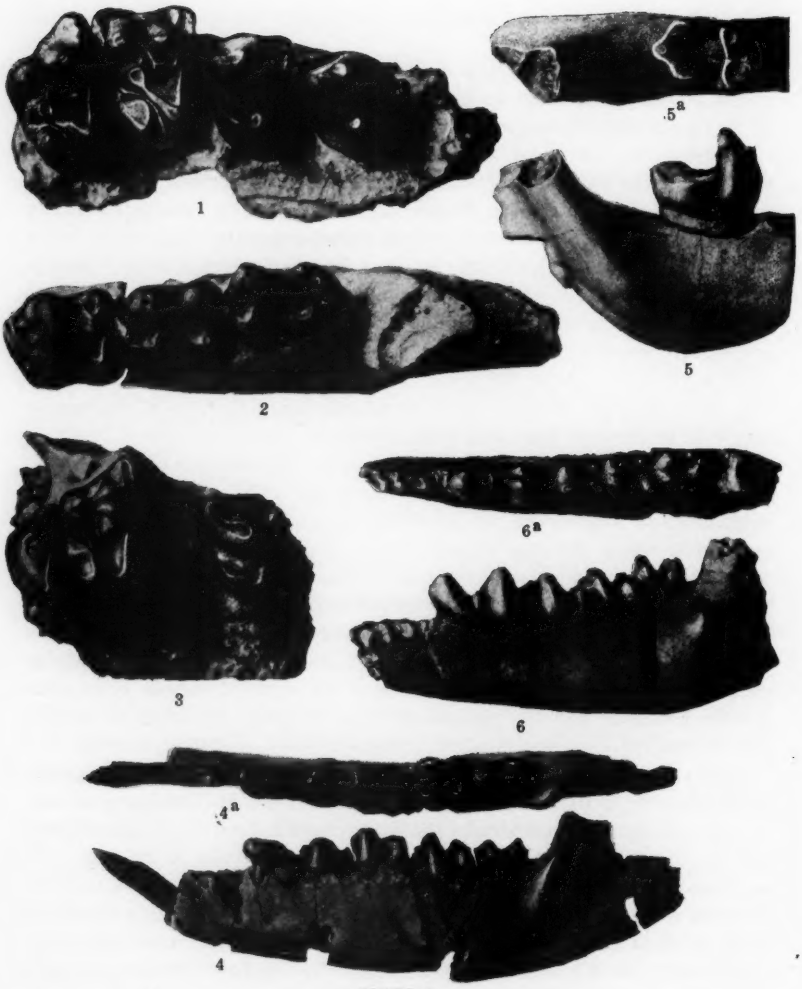


PLATE 1

*Proterixoides davisi*, n. gen. and n. sp.

Figure 1, type specimen, $P\underline{3}-M\underline{2}$, No. 1673, occlusal view; × 5.

Figure 2, $M\underline{1}-M\underline{3}$, No. 1677, occlusal view; × 5.

Figure 3, $P\underline{4}$, $M\underline{2}$ and $M\underline{3}$, No. 1675, occlusal view; × 4.

Figures 4, 4*a*, paratype, No. 1676, mandibular ramus with portion of lower dentition, lateral and occlusal views; × 2½.

Leptictid, probably n. gen. and n. sp.

Figures 5, 5*a*, No. 1679, fragment of mandibular ramus with $M\overline{3}$, lateral and occlusal views; × 3½.

*Sespedectes singularis*, n. gen. and n. sp.

Figures 6, 6*a*, type specimen, No. 1785, ramus of mandible with portion of lower dentition, lateral and occlusal views; approx. × 5.

California Institute of Technology Collections. Sespe Uppermost Eocene, California.

$M\underline{3}$ is smaller, broader anteroposteriorly for its transverse width in *Proterix* than in *Proterixoides* and shows on its crown only the three major cusps. In the Sespe genus the metacone is considerably reduced in size in comparison to the paracone, whereas in *Proterix* the two cusps are of subequal size. The presence of the intermediate cuspules in $M\underline{3}$ of the Sespe genus has been mentioned above.

The anterior opening of the infra-orbital canal lies above $P\underline{3}$.

A number of lower jaw specimens are available, of which the paratype, No. 1676, offers the best opportunity to ascertain the number and character of the anterior teeth. All of the teeth anterior to $P\overline{3}$ are crowded. Evidently three lower incisors were present, although only the crown of $I\overline{2}$ is now preserved. This tooth is distinctly larger than either $I\overline{1}$ or $I\overline{3}$ and its attitude is proclivous. The alveolus for the canine is noticeably larger than that for the last incisor. Two root-sockets can be discerned between the alveolus for the canine and that for $P\overline{3}$, a small one in front and a larger one behind. These are interpreted to represent the position of a two-rooted $P\overline{2}$, although it is possible that each alveolus may be for a single-rooted tooth, namely $P\overline{1}$ and $P\overline{2}$.

In the paratype of *Proterixoides* the inferior border of the ramus is convex in its sweep from a point below the masseteric fossa to the anterior end. The fossa is deep and its upper anterior border, where preserved, is sharply ridged. A posterior mental foramen is situated below the middle of $P\overline{3}$ while a second opening lies slightly farther forward, below the anterior border of $P\overline{3}$.

*Proterixoides* differs from *Diacodon* in presence of a non-molariform $P\underline{4}$, distinctly different shape of $P3$, and in the larger molar teeth with $M\underline{1}$ and $M\underline{2}$ relatively longer anteroposteriorly in comparison to their width. Moreover, the hypocone is larger in these molars and the principal external cusps are farther removed inwardly from the external border of the teeth. In the lower molars the trigonids are lower and less compressed. In $P\overline{4}$ the heel is considerably more reduced than in *Diacodon*. $P\overline{3}$ is without a basal cuspule, but possesses an inner style or ridge.

*Relationships.*—Among known fossil forms *Proterixoides* appears to be most closely related to the Oligocene genus *Proterix*. Resemblance to the latter type is greater than that which prevails between the Uppermost Eocene genus and the Old World erinaceids of the Miocene and Oligocene. Although regarded as ancestral to *Proterix*, the Sespe genus does not serve to bring the erinaceids and the Leptictidae more closely together than does the former type. Characters displayed by the posterior premolars may be taken to indicate an approach toward the Tupaioidea. Resemblance to the type of dentition seen in *Anagale* is apparent, but in the face of the present inadequate basis of comparison an extended statement of possible relationship to the Anagalidae seems hardly warranted.

COMPARATIVE MEASUREMENTS (IN MILLIMETERS)

| | *Proterixoides Davisi* Type No. 1674 C. I. T. | *Proterix Loomisi* Type No. 9756 A. M. N. H. |
|---|---|---|
| Length from anterior end of $P\underline{3}$ to posterior end of $M\underline{3}$ | *a* 13.3 | *a* 14.4 |
| Length of molar series | 8.1 | 8.3 |
| $M\underline{1}$, transverse diameter | 3.6 | 5 |
| $M\underline{1}$, anteroposterior diameter | 4.5 | 3.3 |
| $M\underline{2}$, greatest transverse diameter | 3.8 | 4.9 |
| $M\underline{2}$, anteroposterior diameter | 2.8 | 3.1 |
| $M\underline{3}$, greatest transverse diameter | 2.9 | 3.5 |
| $M\underline{3}$, anteroposterior diameter | 2 | 1.8 |
| $P\underline{3}$, transverse diameter | 3 | 2.9 |
| $P\underline{3}$, anteroposterior diameter | 3.5 | ... |
| $P\underline{4}$, transverse diameter | 3.9 | 4.1 |
| $P\underline{4}$, anteroposterior diameter | 3.3 | 3.5 |
| | Paratype | |
| Length from anterior end of $P\overline{3}$ to posterior end of $M\overline{3}$ | No. 1676 *a* 16.4 | |

*a* Approximate.

### Sespedectes singularis, n. gen. and n. sp.

*Type Specimen.*—No. 1785 Calif. Inst. Tech. Coll. Vert. Pale., incomplete left ramus with $P\overline{3}$–$M\overline{3}$, plate 1, figures 6, 6*a*.

*Characters.*—Differs from Old World Tertiary erinaceids in more nearly equal size of first and second molars, more reduced paraconid, and presence of hypoconulid in $M\overline{3}$. Differs from *Proterixoides* in much smaller size, $P\overline{3}$ more reduced in size, in relation to $P\overline{4}$, and with inner posterior cuspule or ridge on principal cusp absent; $M\overline{3}$ more reduced in size, relative to size of anterior molars.

*Remarks.*—This tiny insectivore from Locality 150 is distinctly smaller than *Proterixoides* and decidedly less abundant in the collections. $P\overline{3}$ in *Proterixoides* is only slightly smaller than $P\overline{4}$ and its crown carries a tubercle or ridge on the postero-internal side of the principal cusp, a backward continuation of which extends to the inner posterior corner of the tooth. In *Sespedectes*, on the other hand, $P\overline{3}$, in relation to $P\overline{4}$, is more reduced in size, the postero-internal ridge or tubercle is lacking, and the heel has a somewhat different appearance. Furthermore, $P\overline{4}$ differs not only from the comparable tooth in *Proterixoides* in basal outline but also in the fact that the highest point on the posterior rim or heel is reached on the inner side of the tooth. In the larger form the highest point is reached nearer the median anteroposterior axis.

The cheek-teeth are crowded and the trigonid of the individual molar overlaps the posterior end of the tooth in front. $M\overline{3}$, relative to the size

of $M\overline{2}$, is a smaller tooth than in *Proterixoides*. It resembles the latter in the presence of a hypoconulid. Several alveoli are shown in the jaw anterior to $P\overline{3}$ indicating that the anterior teeth were crowded. In addition to the two root-sockets presumably for $P\overline{2}$, three alveoli are present, and a full complement of anterior teeth was apparently not represented. A single mental foramen is shown, situated below $P\overline{2}$.

In the type of *Entomolestes grangeri* from the Bridger the molars have the paraconid much more prominently developed than in the Sespe genus; summit of the entoconid in the molars is more marginally situated; $M\overline{3}$ placed farther forward with reference to the anterior border of the ascending ramus; the mental foramen situated farther posteriorly.

Length from anterior end of $P\overline{3}$ to posterior end of $M\overline{3}$ in No. 1785 is 7.3 mm.

**Leptictid, probably n. gen. and n. sp.**

*Specimen.*—Small fragment of right ramus with $M\overline{3}$, No. 1679 C.I.T. Coll. Vert. Pale., plate 1, figures 5, 5*a*.

*Description.*—Unfortunately, this type is known only by the specimen indicated above. The jaw was evidently larger and heavier than in *Ictops* or in *Protictops*. The last lower molar resembles the comparable tooth in lepticids in the high, anteroposteriorly compressed trigonid and in the shape of the talonid. This tooth is considerably larger than $M\overline{3}$ of *Ictops* and presumably differed in this regard also from the comparable tooth in Peterson's genus *Protictops* from the Duchesne River horizon. The paraconid is small, low, and situated on the middle anteroposterior axis of the tooth. A short cingulum is present along the anterior base of the trigonid. The heel is noteworthy because of its length. Judging from the characters of the heel in the anterior molars of *Protictops*, the talonid in the last molar probably was considerably shorter than in the tooth of the Sespe genus. The cusps on the heel in No. 1679 are worn, but it is evident that the hypoconid was a more robust cusp than the entoconid and situated not so far posteriorly as the latter. The hypoconulid has a position on the middle anteroposterior axis of the tooth.

In the characters of $M\overline{3}$ the Sespe form shows greater resemblance to *Diacodon* and to *Leptictis* and *Mesodectes* than to *Ictops* or *Protictops*.

Length of $M\overline{3}$ in No. 1679 is 4.1 mm.

# APPLICATION OF CHAIN-DEFORMATIONS TO CRITICAL POINTS AND EXTREMALS

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*Introduction.*—We have recently developed[1] a general theory of chain-deformations and their applications to locally connected sets and neighborhood retracts in the sense of homology (= respectively *HLC* sets, *HNR* sets). This theory may be said to include the parts of algebraic topology related to local connectedness and retracts and to leave out the rest. In the present note we shall describe the application of the convenient and intuitive machinery developed to the classification of critical points of functions due to Morse.[2] We obtain thus a strictly topological and all-inclusive theorem on this question. We shall also indicate the simplifications which these new tools afford in Morse's applications to extremals.

1. Let at first $\mathfrak{R}$ be a metric space and let $\mathfrak{C}$ be a class of chains forming an additive group, closed under the operation $F$, and whose elements $c_p$ are each on a compact subset of $\mathfrak{R}$. If we wish we may assume that $\mathfrak{C}$ consists of singular chains as usually understood, or of regular chains in the sense of DJ No. 3. In any case we have homology groups, etc., within $\mathfrak{C}$ and it is their homologies which are implicit throughout. Let now $f(x)$ be a continuous single-valued function of the point $x$ of $\mathfrak{R}$ whose values are real numbers $\geqq a > -\infty$. What can be said regarding the variation with $y$ of the homology structure of the sets: $f(x) < y$ or $U^y$, $f(x) \leqq y$ or $A^y = \overline{U^y}$? To make the problem manageable we must impose a certain local regularity on the sets, and hence on $\mathfrak{R}$. We choose the following:[1]

I. *Every $A^y$ is compact and HLC.*

II. *Every $U^y$ is chain-shrinkable away from the set $B^y: y = f(x)$, on $A^y$.*

Roughly speaking these conditions may be said to embody the regularity part of our General Manifold postulates[3] which do not imply any local duality.

We call $y$ a *critical* value whenever there exist non-zero homology groups of $A^y$ mod $U^y$. To such values there correspond closed critical sets $G^y$. The Betti numbers $R_p(A^y, A^y - G^y)$ if $\mathfrak{C}$ admits any, are the corresponding *type numbers*,[4] and $\Delta M_p = R_p(A^y, U^y)$ is the $p$-th type number for $y$, which is necessarily finite. The critical values are isolated, and if two of them $y_1$, $y_2$ are consecutive, the homology groups of $A^y$, $y_1 \leqq y < y_2$, and of $U^y$, $y_1 < y \leqq y_2$ are all identical. From this we deduce readily Morse's relations for the type numbers $M_p = \Sigma \Delta M_p$ (loc. cit. Ch. VI).

In the simplest case: dim $\mathfrak{R} = n$ is finite, $a \leqq f(x) \leqq b < +\infty$, hence $\mathfrak{R}$ is compact and we have between the $M$'s and the Betti-numbers $R_p$ of $\mathfrak{R}$:

$$(-1)^k \Sigma (-1)^p (M_p - R_p) \left\{ \begin{matrix} \geqq 0, & k < n \\ = 0, & k = n \end{matrix} \right\}, \quad (1) \quad (2)$$

$$M_p \geqq R_p. \quad (3)$$

If $\mathfrak{R}$ is not compact but every $M_p$ is finite for $p < n$, (1) still holds. All this remains true if we replace I by:

III. *Every $A^y$ is chain-shrinkable on itself onto a compact subset $\mathfrak{A}^y$ which is HLC.*

If the system of coefficients for $\mathfrak{C}$ is a field and

IV. *The total critical set is HLC*, then the total critical set for any critical value $y$ has a finite Betti-number $R_0$ (DJ No. 16, Th. VIII, also end of No. 18) and hence *it consists of a finite number of connected subsets.*

From the results just quoted it follows also that the Betti-numbers of the sets $A^y$, $U^y$, when any occur, are all finite. Furthermore whenever we affirm that (1) or (2) holds we imply that the numbers $R_p$ in the relation are finite. Finally when $R_p = \infty$ we also have $M_p = \infty$.

In the simplest analytical cases our results are readily shown to include those of Morse.

2. *Application to Extremals.*—It is here that the outstanding application of the critical point theory is to be found so far. The most general topological situation envisaged by Morse (loc. cit. Chs. VII, VIII) refers to any one of the typical problems of the Calculus of Variations when the carrier set is a compact metric topological $M_n$ with local coördinate systems which are mutually analytical wherever they overlap. The open or closed curves, as the case may be, of the maximal conceivable family form a space $\Omega$ metrized à la Fréchet. Those curves $P$ which consist of broken extremals of integral-length $\leqq$ a certain $\rho$, and with less than say $r$ sides, form a compact subspace $\Omega^r$ or $\Omega$ which is *locally polyhedral* (DJ No. 21), and such that $\Omega^r \subset \Omega^{r+s}$. We now consider only regular chains each of which is on an $\Omega^r$ and homologies between these.

Observing that: (a) $\rho$ may be replaced by any $\rho' < \rho$; (b) essentially $P$ is uniquely determined by its sequence of vertices; it is a simple matter to show that if $\{\Omega^r\}$, $\{\Omega''\}$ are two separate aggregates of sets $\Omega^r$ in $\Omega$, the associated homology-classes are simply isomorphic. Hence their homology characters are topological invariants of $\Omega$.

Since the chains are determined by countable sets on an $\Omega^r$, one merely needs to deform isolated extremals, thereby greatly simplifying the treatment throughout.

[1] S. Lefschetz, "Chain Deformations in Topology," forthcoming in the new *Duke Math. Jour.* (= *DJ* in the sequel).

[2] M. Morse, "Calculus of Variations in the Large," Coll. Lect. vol. 18.

[3] S. Lefschetz, "On Generalized Manifolds," *Am. Jour. of Math.*, **55**, 487 (1933).

[4] This definition has already been exploited, on our suggestion, by A. B. Brown, *Ann. Math.*, **32**, 513 (1931).

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## *NOTE ON PONTRJAGIN'S TOPOLOGICAL THEOREM OF DUALITY*

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1. Several extensions have been proposed of the theorem on the duality between the $r$-dimensional Betti group of a complex $C$ immersed in Cartesian $n$-space and the $(n - r - 1)$-dimensional Betti group of the residual space $S - C$. However, the first completely satisfactory treatment of the general case, where the immersed space $C$ is allowed to be an arbitrary closed point set in $S$, has only recently been given by Pontrjagin.[1] The success of Pontrjagin's method depends essentially on the use of generalized chains and cycles, where the coefficients of a chain are no longer required to be integers but may be the elements of an arbitrary abelian group. If the coefficients of the chains of $S - C$ are the elements of any countable, discrete, abelian group $G$ and the coefficients of $C$ the elements of the topological group $H$ composed of all the characters of $G$, then Pontrjagin establishes the elegant duality relation that the $r$-th Betti group of $C$ is homeomorphic with the character group of the $(n - r - 1)$-th Betti group of $S - C$.

Pontrjagin's relation applies to an arbitrary compact metric space $C$ of finite dimensionality immersed, as it always can be, in a Cartesian $n$-space of finite dimensionality. The purpose of this note is merely to point out that we need not make the restriction that $C$ be of finite dimensionality, but may extend the relation to a perfectly general compact, metric space immersed, this time, in a Cartesian space of $\aleph_0$ dimensions. The proof will be sketched very briefly, as the only new idea is the one of introducing cycles of dimensionalities $\infty - r$ in the residual space $S - C$. These cycles were used by me a number of years ago in a course of lectures on topology to prove a much simpler relation of duality between the $r$-th Betti numbers, modulo 2, of a closed point set in Cartesian $\aleph_0$-space and the $(\infty - r - 1)$-th Betti numbers of the residual space.[2] They play the same rôle as the "pseudo cycles" of Lefschetz which the latter developed independently at about the same time in connection with his investigations on transformations and their fixed points.[3]

2. Let $C$ be an arbitrary compact, metric space. Since the space is both metric and compact it must be separable; therefore there exists a countable set of points $p^{(1)}, p^{(2)}, p^{(3)}, \ldots$ everywhere dense on $C$. Now, suppose we denote the distance of a variable point $x$ of $C$ from the point $p^{(i)}$ by $x_i$. Then we may regard the numbers $x_1, x_2, x_3, \ldots$ as the coördinates of a point $x$ in a Cartesian space $S$ of $\aleph_0$ dimensions and think of the space $C$ as immersed in $S$. Clearly a sequence of points $x^{(i)}$ of $C$ will converge to a limit point $x$ if, and only if, each coördinate $x_s^{(i)}$ of the variable $x^{(i)}$ converges to the corresponding coördinate $x_s$ of $x$,

$$\lim_{i=\infty} x_s^{(i)} = x_s, \qquad (s = 1, 2, \ldots); \tag{1}$$

we shall, therefore, topologize the space $S$ by taking Condition (1) as the condition for convergence to a limit in $S$. It is well known that the space $S$ can be metrized by the Fréchet metric

$$D(x, \bar{x}) = \left[\sum_{s=1}^{\infty}\left(\frac{1}{s}\,\frac{|x_s - \bar{x}_s|}{1 + |x_s - \bar{x}_s|}\right)^2\right]^{\frac{1}{2}}$$

in terms of which we see, at once, that $S$ is homeomorphic with the interior of the Hilbert parallelotope determined by the inequalities

$$|x_s| < \tfrac{1}{s}, \qquad (s = 1, 2, 3, \ldots).$$

Given any positive number $\epsilon$ and any *finite* set of coördinates $x_{s_1}, x_{s_2}, \ldots, x_{s_n}$ we may define the neighborhood $N_x(\epsilon; s_1, s_2, \ldots, s_n)$ of a point of $x$ as consisting of all points $y$ of $S$ such that their $n$ coördinates $y_{s_1}, y_{s_2}, \ldots, y_{s_n}$ satisfy the inequalities

$$|y_{s_i} - x_{s_i}| < \epsilon \qquad (i = 1, 2, \ldots, n). \tag{2}$$

The set of all neighborhoods (2) of all points $x$ will be a fundamental set. We may even restrict $\epsilon$ to rational values and $x$ to points with rational coördinates, all but a finite number of which are zero, in which case we shall obtain a fundamental set composed of a countable number of neighborhoods.

3. Let us now consider any finite set of finite linear forms

$$\sum_s a_{is}x_s + b_i, \qquad (i = 1, 2, \ldots, n), \tag{3}$$

where the coefficients $a_{is}$ and $b_i$ are real and where, in each relation, there are only a finite number ($> 0$) of coefficients $a_{is}$ different from zero. By the *signature* of a point $x$ of $S$ with respect to the forms (3) we shall mean a set of $n$ numbers $(\alpha_1, \alpha_2, \ldots, \alpha_n)$ such that $\alpha_i$ has the value 1, $-1$ or 0 according as the value of the $n$-th form (3) at $x$ is positive, negative or zero. Each point $x$ belongs to a convex set consisting of all points of $S$ with the same signature as $x$. We shall call this convex set a *cell* of dimensionality

$\infty - r$ if there are exactly $r$ linearly independent forms (3) vanishing at the points of the set. The set of all the various $(\infty - r)$-cells ($r = 0, 1, 2, \ldots$), will form a finite *subdivision* $\Sigma$ of $S$.

We shall say that a coördinate $x_s$ is *significant* if it occurs with a non-vanishing coefficient in at least one of the forms (3). The significant coördinates $x_s$ determine a *significant* Cartesian space $S_m$ of some finite dimensionality $m$. Each cell of $\Sigma$ of dimensionality $\infty - r$ intersects $S_m$ in an ordinary cell of dimensionality $m - r$. We shall define the incidence relations among the cells of $\Sigma$ so that they correspond to the incidence relations among the intersections of the cells with $S_m$. Thus, we can develop, at once, a theory of $(\infty - r)$-chains, $(\infty - r)$-cycles and homologies on $\Sigma$. The effect of adding further linear forms to the set (3) will be to transform the subdivision $\Sigma$ into a finer subdivision $\Sigma'$. Each chain of $\Sigma$ can be identified with a chain of $\Sigma'$ in the customary manner. By a chain of the residual space $S - C$ we shall mean a chain belonging to at least one finite subdivision $\Sigma$ and determined by a set of cells having no point of $C$ on any of their interiors or boundaries. If the chain is a cycle it will be said to be homologous to zero in $S - C$ if, and only if, there exists some finite subdivision $\Sigma$ on which the cycle bounds a chain of $S - C$. In terms of cycles and homologies we may define the Betti groups of $S - C$ in the usual way.

4. The *dual* $\Delta$ of a subdivision $\Sigma$ may be constructed in exactly the same way as if the space $S$ were of finite dimensionality. On each cell $A$ of $\Sigma$ we shall choose a point $a$. The dual of each $\infty$-cell $A$ of $\Sigma$ will then be the point $a$ that has been chosen upon $A$; the dual of each $(\infty - 1)$-cell $A$ will be the star-cell formed by joining the point $a$ of $A$ to the duals of the $\infty$-cells incident to $A$; ... the dual of each $(\infty - r)$-cell $A$ will be the star-cell formed by joining the point $a$ of $A$ to the duals of the cells of dimensionalities greater than $\infty - r$ incident to $A$. The dual $\Delta$ of $\Sigma$ will thus be an ordinary finite complex composed of cells of finite dimensionalities. The theory of linkages between the $(\infty - r - 1)$-cycles of $\Sigma$ and the $r$-cycles of $\Delta$ may be carried over verbatim from the ordinary case where $S$ is of finite dimensionality.

5. We shall now take an *infinite* sequence of linear forms (3) subject to the sole restriction that if $F$ and $F'$ are any two mutually exclusive closed sets in $S$ there shall always exist a value of $n$ such that the subdivision $\Sigma_n$ determined by the first $n$ forms of the sequence contains no cell on which there lies both a point of $F$ and a point of $F'$. For example, we may construct the sequence (3) out of all forms of the type

$$x_i + b_i \ (i = 1, 2, 3, \ldots; \ b_i \text{ rational}),$$

arranged in any convenient sequential order. We shall arrange the cells of each subdivision $\Sigma_n$ into two classes, the first composed of all cells

having no point of $C$ on their interiors or boundaries, the second composed of the remaining cells of $\Sigma_n$. The cells of the first class will form a sub-complex $\Sigma_n^*$ of $\Sigma_n$. The cells of the second class will not form a complex, since they may have cells of the first class on their boundaries; nevertheless, their duals will form a complex $\Delta_n^*$. Moreover, the cells of the second class will determine a region $R_n$ containing $C$.

Now, there is no difficulty in extending Pontrjagin's relation of duality to the Betti Groups of $\Sigma_n^*$ and $\Delta_n^*$. Moreover, every cycle of $S - C$ is homologous to a cycle of $\Sigma_n^*$ for sufficiently large values of $n$ and bounds in $S - C$ if and only if the corresponding cycle of $\Sigma_n^*$ bounds for sufficiently large values of $n$. On the other hand, the regions $R_n$ close down on the point set $C$ as $n$ increases indefinitely, in the sense that the intersection of all the $R_n$'s is precisely $C$. Thus, the proof of the relation of duality between the Betti groups of $C$ and $S - C$ may be carried through as if the space $S$ were of finite dimensionality.

[1] L. Pontrjagin, "The General Topological Theorem of Duality for Closed Sets," *Ann. Math.*, **35**, 904–914 (1934).

[2] Cf. the reference in Lefschetz's Topology, *Amer. Math. Soc. Publications*, vol. *XII*, end of p. 315 (1930).

[3] Lefschetz, loc. cit., pp. 341 et seq.

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## *CONNECTIONS BETWEEN DIFFERENTIAL GEOMETRY AND TOPOLOGY*

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In this note are stated the definitions and results of a study of new connections between differential geometry and topology. Proofs will be given elsewhere.

Let $S$ be a complete analytic 2-dimensional Riemannian space (for brevity, complete surface), as defined by H. Hopf and W. Rinow,[1] and $A$ an arbitrary point on it. A point $M$ on a geodesic ray issuing from $A$ is said to be a *minimum point with respect to A on g* if $M$ is the last point on $g$ such that $AM$ furnishes an absolute minimum (proper or improper) to the arc length of curves on $S$ joining $A$ to $M$.[2] We study the locus of such points on all the geodesic rays issuing from $A$.

A geodesic arc is said to be *of class A* if it furnishes an absolute minimum to the arc length of curves joining its end-points. A geodesic ray is said to be *of class A* if every segment of it is of class $A$. The *order* of a minimum point $M$ with respect to $A$ denotes the number of geodesics of class $A$ joining it to $A$.

THEOREM 1. *A minimum point of order* 1 *on g with respect to A must be conjugate to A, and must be a cusp of the locus of first conjugate points to A on the geodesic rays issuing from A.*

Thus the minimum point locus and the conjugate point locus are related, and we study the latter in order to obtain information about the former.

THEOREM 2. *On a complete surface S, the locus of first conjugate points to A is either*

(1) *no points at all,*

(2) *a single point,*

(3) *a closed curve containing a finite number* $\geqq 2$ *of cusps turned toward A and an equal number turned away from A,*

(4) *a set of one or more open curves, each asymptotic to a pair of geodesic rays issuing from A. Each curve of the set contains at least one cusp turned toward A, and one less cusp turned away from A. A single one of the curves cannot contain an infinite number of cusps on a finite segment of the curve. The number of curves in the set can be infinite only if a curve of the set can be found all of whose points are arbitrarily far from A along the geodesics on which they are conjugate to A.*

*In each case the locus can be analytically parameterized in terms of* $\theta$*, the angular coördinate of the geodesic ray from A.*

The following theorem gives a first indication as to how the existence of minimum points with respect to an arbitrary point $A$ of a complete surface is connected with the topology of the whole surface.

THEOREM 3. *A complete surface S is closed* (i.e., *compact*) *if and only if it contains no geodesic ray of class A. It is open* (i.e., *not compact*) *if and only if it contains a geodesic ray of class A issuing from an arbitrary point A.*

THEOREM 4. *Let A be an arbitrary point on the complete simply connected surface S. Then if S is closed, hence homeomorphic to the sphere, the locus m of minimum points with respect to A consists of a single point, or else a tree with a finite number of branches. If m is a single point, it is conjugate to A on all geodesics joining it to A and at equal distances from A along these geodesics. If S is open, hence homeomorphic to the plane, m consists of nothing at all, or a set of one or more distinct trees each of which contains one branch extending infinitely far from A. There may be an infinite number of branches extending from this branch, but not from any bounded segment of it. Whether S is open or closed, the number of arcs of m incident with a point M of m is equal to the order of M as a minimum point with respect to A. An arc of m containing no points conjugate to A and no interior points of order* $> 2$ *is a regular analytic arc. The region S-m-A is simply covered by the geodesic rays through A cut off at their intersections with m, and hence forms a 2-cell with m as its singular boundary, in which the geodesic polar coördinates with A as pole form a coördinate system.*

THEOREM 5. *Let A be an arbitrary point on a closed surface of genus* > 0. *The locus m of minimum points with respect to A is a linear graph* (i.e., *a finite one-dimensional complex*) *whose cyclomatic number is equal to the connectivity number modulo* 2 *of S. If S is orientable, as the geodesic ray from A sweeps out the angle from* 0 *to* $2\pi$ *the minimum point on the ray traces out each one-cell of m once in one sense and once in the opposite sense. Conversely, this is sufficient that S be orientable. Thus the topology of S is completely determined by the linear graph m and the way in which it is traced out. The statements of the previous theorem as to the order of the points of m, the regular analyticity of arcs of m, and the simple covering of the region S-m-A hold here as well.*

This reduction of a closed surface to a single 2-cell with a linear graph as its singular boundary is analogous to the process in analysis situs of making a similar reduction by coalescing 2-cells of a 2-dimensional manifold.[3] Incidentally, these results clear up completely the hitherto vaguely answered question as to how long the geodesics through a point $A$ of a Riemannian surface form a field.

These methods and results present possibilities of generalization to $n$-dimensional Riemannian spaces, to the geometry of paths and to arbitrary convex metric spaces.

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[1] "Ueber den Begriff der vollständigen differential-geometrischen Fläche," *Comm. Math. Helvet*, 3 (1931).

[2] Cf. Blaschke, *Vorlesungen über Differentialgeometrie I*, p. 231, lines 1–6.

[3] See, for example, Veblen, *Analysis Situs*, p. 74, §§ 62, 63.

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## *THE SENSITIVE-VOLUME OF THE MEIOTIC CHROMONEMATA OF GASTERIA AS DETERMINED BY IRRADIATION WITH X-RAYS*

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In studies of the lethal action of x-rays upon cells, measurements of the volume sensitive to x-rays have been subject to a serious error because populations of cells in various stages of division have been used. On the other hand no quantitative measurements of the effect of x-rays on specific stages of cell division have been made. This paper is an account of the results of an experiment designed to detect varying sensitivities of different stages of the chromosome cycle and to determine the order of magnitude of the portion of the chromosome sensitive to x-rays.

The distribution of the various stages of meiosis in a regular sequence in buds along the inflorescence of Gasteria makes possible a study of the effect of irradiation on these stages and on the preceding mitosis. The species used have four long and three very short chromosomes (Marshak, 1934). In this work only the effects induced in the long chromosomes were followed.

The source of x-rays was an air-cooled Coolidge tube with a tungsten target. The primary current was kept at 110 volts, 10 milliamps. The secondary voltage was determined from the spectrum obtained with a spectrograph containing a rock salt crystal. The shortest wave-length was found to be 0.15 Å corresponding to a peak secondary voltage of 82,400 volts. The range of wave-lengths was from 0.15 to 0.40 Å. In the calculations the mean wave-length is taken as 0.30 Å.

The dosage was varied by altering the time of exposure and the distance from the target. Dosage was measured with a Victoreen dosimeter. I am indebted to Dr. J. C. Hudson for the determinations of the characteristics of the radiation used.

The effects of x-rays as observed in the first meiotic anaphase may be put into three groups: (1) chromosome attachments, (2) fragmentation and (3) achromatic spots (similar to secondary constrictions in the chromonema). There is no increase in the frequency of these chromosome abnormalities until somewhat more than an hour has elapsed between irradiation and examination of the cells.

In table 1 the frequencies of the total chromosome abnormalities produced in buds of various ages are given. There are pronounced maxima in buds which reached the anaphase at one and five days after radiation. These buds were in the pachytene and early premeiotic prophase, respectively, when irradiated. In untreated plants 6723 pairs of chromatids were examined at the first meiotic anaphase and showed 0.5 per cent chromosome attachments, 0.02 per cent chromosome fragments and 0.007 per cent (one case) of achromatic spots.

If the frequency of effects produced in buds rayed in pachytene be plotted against dosage, a straight line passing through the origin is obtained as the best fit to the points. A similar relationship is obtained from the values of the second peak (109 to 144 hours after radiation), given by buds irradiated during the premeiotic mitosis. Buds taken at 48 and at 72 hours after radiation also show a linear relationship to dosage. Such a linear function is taken to indicate that photoelectrons produced in the absorption of x-rays within the tissue act directly upon the biological agents involved in the binding together of the constituent portions of the chromonema.

Accepting the hypothesis of direct action, it becomes necessary to explain by means of it the higher frequency of chromosome aberrations in-

TABLE 1

PER CENT TOTAL CHROMOSOME ABNORMALITIES WITH DIFFERENT DOSAGES

| TIME AFTER RADIATION, HRS. | DOSE IN R. 2.6 | 5.3 | 15.8 | 30 | 79 | 264 | 528 |
|---|---|---|---|---|---|---|---|
| 0.5 | | | | | | 0.64 | |
| | | | | | (1.1) | | (0.46) |
| 1 | 1.71 | | | | 1.0 | 0.91 | 0.40 |
| | | | | | | | (73.2) |
| 1.5 | | | | | | | 49.1 |
| 2 | | | | 1.01 | | | |
| | | | | | | (76.9) | (152.6) |
| 24 | | | 2.20 | | 15.9 | 51.4 | 98.1 |
| | | | | (6.5) | | | |
| 36 | | | | 5.3 | | | |
| | | | | (3.4) | (8.8) | | (97.0) |
| 48 | 0.97 | 1.35 | 0.98 | 3.0 | 6.9 | | 61.2 |
| | | | | | | (28.2) | (97.0) |
| 60 | | | | | | 18.5 | 59.2 |
| | | | | (3.05) | (8.4) | | (57.6) |
| 72 | 0.74 | 0.26 | | 2.75 | 6.7 | | 57.0 |
| | | | (0.68) | | | | (67.3) |
| 96 | | | 0.60 | | | | 44.1 |
| | | | | | | | (150.9) |
| 109 | | | | | | | 92.6 |
| | | (1.58) | | | (39.3) | | |
| 120 | | 1.24 | | | 28.4 | | |
| | | | (1.65) | (4.1) | | | (97.8) |
| 144 | | | 1.42 | 3.54 | | | 59.2 |
| | | | | (3.93) | (3.4) | | (13.6) |
| 168 | | | | 2.96 | 2.3 | | 8.96 |
| | | | | (1.36) | | | |
| 216 | | | | 1.05 | | | |
| | | | | (0.71) | (3.5) | | |
| 240 | | | | 0.52 | 2.6 | | |
| 266 | | | | | | | |
| | | | | (1.24) | (1.8) | | |
| 288 | | | | 0.80 | 1.3 | | |
| | | | | (2.45) | | | |
| 312 | | | | 2.00 | | | |
| | | | | | (3.7) | | |
| 336 | | | | | 2.8 | | |
| | | | | (0.81) | | | |
| 424 | | | | 0.61 | | | |

Attached chromatids have been counted as effects produced in pairs of chromatids and achromatic spots as effects in single chromatids. The figures in parentheses are the percentages of chromosome abnormalities when fragments are counted as resulting from effects produced in pairs of chromatids, those not in parentheses are the percentages when a fragment is considered an effect in one chromatid.

duced in cells irradiated at particular stages of division. Such an explanation must apply to the premeiotic mitosis as well as to the synaptene-pachytene stage of the meiotic prophase. Of several schemes devised to indicate the manner in which the condition leading to attached chromonemata might be produced, only one seems to satisfy the conditions adequately. This requires that each synapsed homologue be at least two-parted at the time of radiation, that a change of linkage take place between a pair of strands when a photoelectron intersects both, and that a chromonematic division occur between this stage and anaphase. Careful examination of

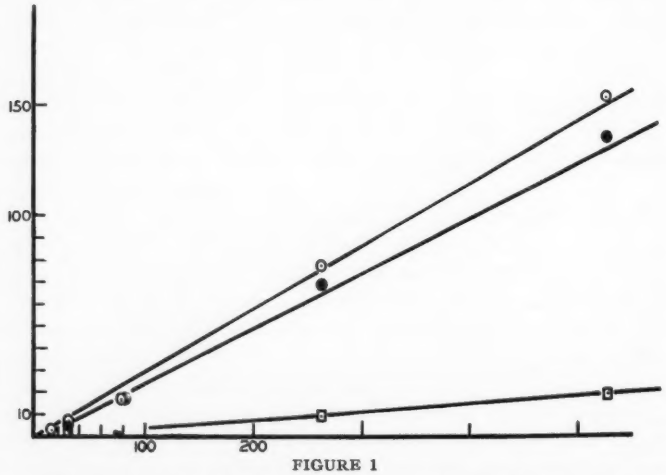


FIGURE 1

The frequency of x-ray-induced chromosome abnormalities observed in anaphase I twenty-four hours after irradiation plotted against dosage in roentgens. These data represent effects induced in the synaptene-pachytene stage of meiosis. Open circles represent frequencies of total chromosome abnormalities, closed circles chromosome attachments plus fragments and rectangles achromatic spots. The first division is considered always reductional. Ordinates = per cent effects, abscissae = dose in roentgens.

anaphase chromosomes from unrayed plants showed that the chromonema of each chromatid was visibly two-parted at least along some portions of its axis. From the same untreated material there are visible indications that the attachment of chromonemata that leads to attenuation of the chromonematic helix due to an interlocking of half-chromonemata. The condition in the x-rayed material is very much the same, and it seems reasonable to suppose that the mechanisms immediately responsible are similar in the two cases. In the irradiated cells there is another type of attenuation in which a portion of the chromonema becomes drawn out to a slender thread

which finally breaks, leaving the distal portion of the chromatid pair on the median plate. Since chromonemata may remain attached through the second meiotic anaphase (as far as they were followed), it seems quite possible we are dealing with two really different conditions. However, any explanation requires that pairs of chromatids must be affected, since the condition observed is usually an attenuation of a portion of both members of a pair of chromatids. The schemes for both types of attachments require the presence of pairs of very intimately associated strands, a condition which may be common to the early prophase of the last premeiotic mitosis and the pachytene stage of meiosis. In the one case (mitosis) the close association may be considered as due to the division of the units of the chromonematic strand forming the new units of the still microscopically undetectable chromonema. In meiosis the condition is produced by synapsis or by another chromonematic division.

An estimate of the sensitive volume of the chromonema was obtained by using the equation given by Glocker (1932) for the number of photoelectron paths traversing a given volume, $N = N_0 \cdot \frac{R + a}{a} \cdot v$. Using $N_0 = 12.4 \times 10^8$, $R = 46 \times 10^{-4}$ cm. and $N = 2.6 \times 10^{-3}$ obtained from the slope of the uppermost line of figure 1, the total sensitive volume of the pachytene chromosome (one of the pair of homologues) is $7.22 \times 10^{-15}$ cm.$^3$ if the first division is always reductional. If the chromatids disjoin at random, then this volume is $20.6 \times 10^{-15}$ cm.$^3$.

By analogy with the *B*-type chromosome in Zea (McClintock, 1933) the reduction in length of the chromonema from pachytene to metaphase was calculated as being about 11 times. Knowing the length of the metaphase chromonema by measurement ($1.93 \times 10^{-3}$ cm.) the length at pachytene is estimated at $21.2 \times 10^{-3}$ cm.

The meiotic nucleus of Gasteria is spherical and has a diameter of $35 \times 10^{-4}$ cm. The number of photoelectrons traversing the nuclear volume may be calculated from Glocker's equation. This may be taken as the number of photoelectrons intersecting the surface of the nucleus. As measured under the microscope the diameter of the pachytene chromosome (bivalent) is $13 \times 10^{-6}$ cm. Assuming a cubical cross-section for the pachytene chromosome it will have an area on one face of $27.6 \times 10^{-8}$ cm.$^2$. Since the number of photoelectrons intersecting the nuclear surface is 82.5 per roentgen, the number of photoelectrons traversing the pachytene chromosome will be approximately $5.9 \times 10^{-1}$ per roentgen. By comparison with the observed frequency of induced abnormalities the width of the sensitive portion of the half chromosome or pair of chromatids can be calculated. With the first anaphase reductional this will have a value of $8.7 \times 10^{-7}$ cm., with random disjunction $12.2 \times 10^{-7}$. If the sensitive volume obtained by the previous method be distributed along the length

of the chromonema the widths obtained are $8.25 \times 10^{-7}$ cm. for reductional division at anaphase I and $13.9 \times 10^{-7}$ for random disjunction. Halving these values gives the diameter of the sensitive portion of the single chromatid: 4.4, 6.1, 4.2, 7.0, $\times 10^{-7}$ cm. Since genetic evidence from Drosophila and Zea indicates that the first division is reductional, the diameters 4.4, 4.2, $\times 10^{-7}$ cm. are the most probable. These agree well with the diameters for small protein molecules albumin = $4.34 \times 10^{-7}$ cm. (Svedberg and Sjogran, 1929; Nichols, 1930), trypsin = $5.2 \times 10^{-7}$ cm. (Northrop and Kunitz, 1932), insulin = $4.36 \times 10^{-7}$ cm. (Sjogran and Svedberg, 1931). The maximum diameters (with random disjunction are considerably smaller than the diameter of the large haemocyanin molecule $2.4 \times 10^{-6}$ cm. (Svedberg and Chirnoaga, 1928).

The sensitive volume of a pair of chromonemata for production of achromatic spots calculated by the second method previously given is $0.9 \times 10^{-15}$ cm.$^3$, for attachments plus fragments it is $7.6 \times 10^{-15}$ cm.$^3$, for total induced abnormalities $8.0 \times 10^{-15}$. If attachments and fragments be considered consequences of effects in the linkage mechanism then this mechanism makes up about 90 per cent of the total sensitive volume. If the linkage mechanism is distributed along the long axis of the chromonema then its reduction to a minimum will reduce the length of the chromonema to about one-tenth of its extended size. This is in good agreement with the reduction in length from pachytene to metaphase of eleven times as previously estimated, and indicates that the contraction of the chromonena from prophase to metaphase is due largely to a reduction in the linear extension of the linkage material.

A more detailed account of these results is being published elsewhere.

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# MOVEMENT OF SENSORY HAIRS IN THE EAR

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The movement of hair cells from the canal of the ear of *Petromyzon marinus* was recorded by Ecker (1844). He found, within the labyrinth, certain cells, each of which bore a single ciliary process, and reported activity upon the part of such processes. There appears to be no mention, however, of whether these motile hairs formed definitely a part of the neuro-epithelial region of the crista, or came from other parts of the membranous labyrinth.

In previous reports (Bowen, 1931, 1932), I have described the movement of sensory hairs from the ear of the common horned-pout, *Ameiurus nebulosus*. Such movement was witnessed in some, though by no means all of the preparations which showed the crista in fresh condition. This activity proved usually to be a definite, whip-like lashing, or wave-like motion in which the whole hair, from base to tip, was involved. In one of the ampullae from *Ameiurus*, hairs were found to be beating in such unison as to suggest some form of coördination. No indication was noted of any definite length of time during which motion might continue, nor did there seem to be any regularity as to periods of movement and of rest.

Investigations have recently been made of the ampullar organs of two Bermuda fishes, the squirrel fish, *Holocentrus ascensionis*, and the blue-striped grunt, *Haemulon sciurus*. These marine teleosts are both common in the Bermuda waters. It was found possible, by employing methods which have been noted in my earlier reports, to see the sensory hairs of the crista clearly and to hold them in living condition for some hours.

In one preparation from the squirrel fish, definite movement of the so-called sensory hairs was observed. The hairs in this case were apparently undisturbed by the dissection, and seemed, in all detectable respects, to be a part of uninjured tissue. The activity of the hair cells which was observed in this crista from the squirrel fish was somewhat less than I have witnessed in preparations from the *Ameiurus nebulosus*. There were no vigorous lashings here, though the amplitude of motion was wide enough to render the fact of movement itself clear and unmistakable. The crista had been kept in saline solution sufficiently long that no diffusion currents could be held responsible for the motion, and the preparation was so mounted that other currents, such as those due to movement of air in the vicinity of the preparation, were avoided.

In this crista, several hairs were moving, but they were not so situated as to make possible a determination as to coördination. The rate of move-

ment was less rapid than I have witnessed in *Ameiurus*, being here on the order of 125 beats per minute, as compared with a maximum of near 300 in the catfish.

In two dissections from the blue-striped grunt, movement of sensory hairs was observed. In one of these preparations, two hairs only were moving and, again, not with the activity shown by some specimens from *Ameiurus*, but with a wave-like movement still sufficient in amplitude to show that the movement was on the part of the hairs themselves. The arc through which the tip of the hair moved was somewhat more than half the length of the process itself.

In another crista from the blue-striped grunt, a distinct lashing of a single hair was observed. This activity occurred at the base of the proximal side of the mound of neuro-epithelial tissue which, in this species, is found near the center of the crista. This action continued for about ten minutes from the time it first came under observation, movement in this case being a distinct lashing in which the whole hair, from base to tip, participated.

These observations, one group from a fresh-water teleost, and the other two from marine forms, indicate that the phenomenon of movement of the so-called hairs within the ear is not limited to a single species and, it is suggested, may be found throughout a wide range of animal forms. In so far as I have record, there has been no mention of movement on the part of the sensory processes of the ear in any class above the fishes.

* Investigations on the ampullar organs of certain marine fishes in Bermuda were begun in 1931, and were made possible through a grant from the James F. Porter Fund, Harvard University.

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